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INVERTEBRATE NUTRITION. I. A PRELIMINARY SURVEY OF THE NUTRITIONAL REQUIREMENTS OF AN ISOPOD: *ONISCUS ASELLUS**

ERNEST BEERSTECHER, JR., JOHN CORNYN, CAROL VOLKMANN, LAVERNE CARDO, AND RITA HARPER

The need for extended investigation of the nutritional requirements of the many animal groups falling phylogenetically between the protozoa and the insects has recently been emphasized (Beerstecher, 1950). Because of their economic importance, the crustaceans seem particularly deserving of study in this regard. Almost nothing is known with certainty concerning crustacean nutrition (Beerstecher, 1952). This subphylum is a large one, showing great morphological variety, so that nutritional data concerning any single group might well not represent the entire membership of crustacea. Nutritional studies of a number of markedly different species representative of different classes and orders of the subphylum have therefore been undertaken. In each instance the major problem has centered around the development of suitable experimental techniques for the investigation of this essentially aquatic group. This present report summarizes the results of a preliminary survey of the nutrition of a representative member of the order of isopods, *Oniscus asellus*, more commonly known throughout the United States as the pillbug, because of its armadillo-like appearance which permits it to roll up into a small ball when touched. This species represents an economic problem in most truck gardening areas because of its feeding habits. Subsequent reports will deal in greater detail with more specific aspects of its nutrition.

Experimental

The common pillbug found in the United States seldom exceeds 125 mg. in weight, is born alive from an egg pouch on the belly of the female, and at birth weighs in the order of 0.1 mg. Weight is a satisfactory criterion of growth in the young (those weighing less than about 30 mg.), but growth is rather slow in larger animals. The appearance of molts provides a criterion

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of development, and survival under various experimental conditions may be used as an indication of nutritional sufficiency. The pillbug is omnivorous and cannibalistic. Its respiration requires a moist environment.

The animals used in this study were found on the soil in the vicinity of Houston, Texas, and stocks were maintained in large battery jars containing moist earth. Moisture was maintained by inverting in the earth a bottle filled with water. Experimental groups of five animals each were maintained in 4 oz. ointment jars, containing in the bottom a moist layer of washed white sand. A small hole in the lid of the jars permitted extension through the lid of a four-inch test tube filled with water and inverted into the sand to maintain the moisture. The experimental diets were placed on one-inch square aluminum foil pallets, which were laid on the sand. Cages and diets were changed, and animals individually weighed, twice weekly. At these times observations were also made of the presence and appearance of feces, molts, or fungi, and molts were also weighed. The animals were maintained at temperatures of from 65–75° F.

Diets were made up in the usual manner of preparing synthetic diets for rats or other laboratory animals, and were kept in sealed jars at 4° C. All diets employed U.S.P. salt mixture No. 2 as the mineral supplement, and powdered cellulose acetate (Alphacel) as a material to provide bulk. The basic diet consisted of: vitamin free casein, 20 per cent; salt mixture, 5 per cent; starch, 10 per cent; cellulose acetate, 59 per cent, and Wesson oil, 6 per cent.

To this was added, unless otherwise indicated, 400 mg. ascorbic acid and .5 ml. U.S.P. cod liver oil per 100 gm. of diet. A basic B vitamin supplement, added in amounts of 900 mg. per 100 gm. of diet when indicated, had the following composition: thiamin chloride HCl, 100 mg.; riboflavin, 100 mg.; nicotinic acid, 200 mg.; calcium pantothenate, 200 mg.; pyridoxin, 50 mg.; biotin, 50 mg.; folic acid, 50 mg.; p-aminobenzoic acid, 500 mg.; choline HCl, 500 mg.; inositol, 500 mg., and vitamin B_{12}, 10 μg.

Groups of animals were studied on diets modified to contain 0, 3, 10, and 20 per cent protein lacking the B vitamin supplement, but with and without 1 per cent Difco yeast extract; diets modified to contain no fat, with and without the vitamin supplement; and modified to be free from each of the members of the B vitamin supplement. Other diets lacked vitamin C or vitamins A and D. Animals were also studied on diets containing only cellulose acetate, and in the absence of all food.

Results

About three-quarters of all animals tested in the absence of food survived for a period of 45 days or longer, during which, however, they lost an average of 12 per cent (range: 5 to 25 per cent) in weight. Such a weight loss for a relatively hard shelled animal is of course extreme. The ability to survive for this period in the absence of food must be taken into account in the results that follow. Animals on a diet of cellulose acetate showed a simi-

lar ability to survive, but an average weight loss of only 4 per cent (range: 0–12 per cent). Survival on this diet drops to about 40 per cent in 90 days, and few animals survive much longer than this.

The results obtained by modifying the protein content of the diet are summarized in Table 1. Maximum growth rate occurs at

TABLE 1

Effects of Dietary Protein Level on Oniscus asellus

Percent protein in diet	Animals per group	Percent survival (75 day test period)	Average percent weight change	Percent animal days survived*
0	30	60	— 4	75
3	10	80	— 3	93
10	10	80	+ 6	83
20	10	40	0	60

*Sum of life span of individual animals divided by product of animals per group and length of test.

about 10 per cent protein, 3 per cent being inadequate, and 20 per cent apparently toxic. Survival is also best in the range from 3–10 per cent. These results are modified somewhat by the presence or absence of added growth factors in the form of yeast extract. Added to the protein free diet groups, yeast extract increased the weight change from an average loss of 5 per cent to a gain of 5 per cent, but it seemed to decrease survival. In the 3 per cent and 10 per cent protein groups both weight gain and survival were improved by the yeast, while only a mildly deleterious effect on survival could be noted in the 20 per cent protein diet groups. Maximum weight gain and survival occurred in the presence of 10 per cent protein and yeast, and typical growth curves are shown in figure I. Also shown are growth curves from animals on the cellulose acetate diet.

Because of the prolonged survival on protein free diets and the absence of extreme weight losses, further attempts were made to explore this phenomenon. Repeated chemical analysis of the diet by the Kjeldahl method showed it to contain less than 0.1 per cent nitrogen. Animals were then fed on diets containing 1 per cent sulfapyridine added to the 0 and 3 per cent protein diets plus the B vitamin supplement. This measure caused an average loss in weight in the protein free diet animals of 12 per cent, as

contrasted to a gain of 5 per cent in the absence of the drug. The sulfapyridine did not appreciably affect the growth of animals on the 3 per cent protein diet, however, nor did it affect survival in either group. These observations strongly suggested that the intestinal microflora of these animals might be involved in the phenomenon. *Oniscus* usually consumes considerable quantities of dirt in its normal habitat, and might be expected to contain intestinal nitrogen-fixing bacteria initially, but it was uncertain whether these would survive on nitrogen free diets over a period of time. A number of animals were therefore washed with bichloride of mercury and the contents of their gut cultured. It was found that all contained large numbers of organisms which on the basis of morphology and physiology were indistinguishable from *Azotobacter agilis*. The organisms were capable of luxuriant growth in a nitrogen free medium. Symbiotic relationships between nitrogen-fixing bacteria and *Arthropoda* have previously been reported. Confirmation of this relationship by the use of isotopic nitrogen studies is planned, but has not as yet been undertaken in our laboratories.

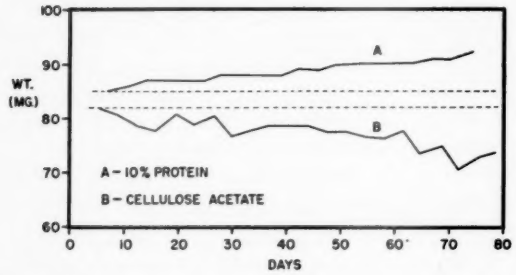

Figure I. Typical growth curves of pillbugs on 10 per cent protein and on cellulose acetate diets.

Modification of the fat content of the diet, or the content of vitamins A, C, or D had no appreciable effect upon survival or growth rate. It was therefore concluded that these substances are not dietary essentials for this species. Vitamin 'C,' if present in the animal, is only found in minute amounts not detectable by standard titration procedures, and feeding animals on high ascorbic acid foods (lemons, green peppers) does not increase the amount. Supplementation of the basic diet with the synthetic

B vitamin supplement rather than yeast extract gave results almost identical with those obtained with the yeast, although slightly better growth was obtained. Omission from the diets of individual animal groups of the various components of the B vitamin mixture produced erratic but probably non-significant changes in growth, and it is concluded that the intestinal flora is generally sufficiently vigorous to supply these factors in amounts sufficient for growth. Microbiological assay of animals maintained on the riboflavin deficient diet showed that these animals contained quantities of riboflavin comparable with those found in normal animals (16–26 μg./gm. wet weight).

Discussion. The results of this survey study on the nutrition of *Oniscus* bear out what might be expected on the basis of previous knowledge for an animal of this type. Protein in moderate amounts has a salutory effect on growth and survival, but apparently becomes toxic at higher levels. Vitamins A, C, or D are not essential for growth, nor are fats. Intestinal flora furnish the B vitamins required, but added amounts promote further growth in the presence of suitable protein levels. In the absence of dietary protein they seem to stimulate the assimilation of other nutrients, but concurrently cause effects that decrease survival. The data suggest the possibility that this animal may obtain fixed nitrogen through the agency of symbiotic intestinal bacteria, but further confirmation of this observation is required.

Summary. Nutritional studies were conducted on the isopod, *Oniscus asellus*, and results covering some 25 groups of animals are reported. The data suggest nutritional requirements similar in general to those of some insects.

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INVERTEBRATE NUTRITION. II. THE EFFECTS OF VITAMIN AND AMINO ACID ANALOGUES ON ONISCUS ASELLUS*

ERNEST BEERSTECHER, JR., JOHN CORNYN, AND CAROL VOLKMANN

In the preceding report it was shown that the activities of intestinal microorganisms are of such a magnitude in *Oniscus asellus* that only limited data concerning nutritional requirements may be obtained by growing these animals on deficiency diets. For this reason, studies have been conducted on this species grown on synthetic diets containing various analogues of vitamins and amino acids. The results of these studies are reported here, and they show that in a few instances, at least, nutritional behavior of the analogue is quite similar to that which has been obtained with higher animals.

Experimental

The techniques employed are those previously described. Vitamin analogues were employed with the basic diet containing the vitamin supplement but lacking the appropriate vitamin for which the analogue was designed. Amino acid analogues were used with the complete vitamin supplement diet containing 20 per cent casein.

Results

A number of analogues at levels of 1 per cent in the diet were without any apparent effect on growth or survival. These are listed in table 1. With a number of others, however, more promising results were obtained. One per cent methyl 3-pyridylketone, an analogue of niacin, decreased survival to about 30 days (from over 90 days), but produced only a slight weight loss. The effect was not reversed by niacin. This analogue has been reported to affect other species in a similar manner. Diets containing 1 per cent beta-2-thienylalanine, an analogue of phenylalanine, cut the average survival of experimental animals to 40 days and

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caused a weight loss of 5 per cent, despite the presence of phenylalanine in the casein of the diet. Addition of 0.5 per cent DL-phenylalanine to the diet restored survival to normal, but had

TABLE 1

Analogues Showing No Inhibitory Effect on Oniscus Asellus at the 1 Percent Level in the Diet

Nutrient	Analogue
Niacin	3-Pyridinesulfonic acid
Pantothenic acid	Pantoyltaurine
	Methylpantothenic acid
Pyridoxine	Desoxypyridoxine
Biotin	Desthiobiotin
p-Aminobenzoic acid	Sulfapyridine
Tyrosine	Aminotyrosine

little effect on the weight. One per cent DL-phenylserine, another analogue of phenylalanine, had little or no effect on weight or survival. Addition to this, however, of 1 per cent DL-phenylalanine resulted in a tremendous average weight loss of about 20 per cent, greater than that of animals on a starvation diet, as shown in figure I. The phenomenon is being investigated further.

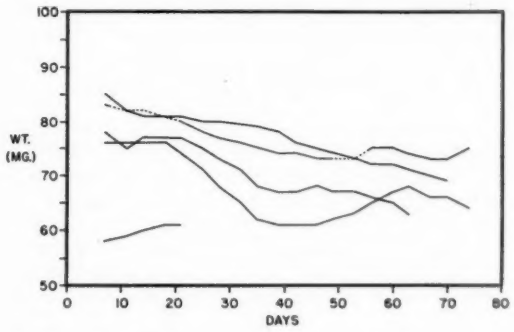

Figure I. Growth curves of pillbugs on the 1 per cent phenylserine—1 per cent phenylalanine diet.

Studies were also undertaken with diets containing 2 per cent DL-ethionine, an analogue of methionine. Survival was greatly depressed to about 28 days, while weight was not affected in this brief period. Addition of 0.5 per cent, 1 per cent, and 2 per cent

methionine to the diet increased survival to 32, 41, and 46 days respectively. Betaine does not reverse the inhibitory effects of this substance as it does in some higher species, nor is methionine completely effective. Studies on complete serial sections of animals from the major groups studied are being undertaken, for comparison of the observations with the pathological lesions produced under similar circumstances with higher animals.

Discussion. While the use of inhibitory analogues of metabolites in nutritional studies has seldom resulted in any conclusive information regarding exact nutritional requirements, their employment has often given confirmatory evidence and indications of possible relationships. In the present instance, the analogues of niacin and methionine produced results in a crustacean that are very similar to those seen in many higher animals, as well as in microorganisms. Inhibitory action by phenylserine itself was unimpressive, but the synergistic inhibition brought about by phenylalanine with phenylserine would seem to be of interest. In this case it seems entirely possible that the two aromatic amino acids act cumulatively to block some enzyme system involved with another group of substances, in a manner similar to the inhibition of phenylalanine metabolism in bacteria caused by high levels of tryptophan (Beerstecher, 1947). The lack of activity of a number of analogues that have been shown to be quite effective with other species again suggests the existence of a high level of synthesis of metabolites in the gut of this animal.

Summary. The effects of eleven inhibitory analogues of vitamins and amino acids on the growth of a crustacean, *Oniscus asellus*, are reported, and the results interpreted in terms of probable nutritional requirements for this species.

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MAMMALIAN HYPOTHALAMIC NUCLEI CULTIVATED *IN VITRO*

E. Borghese*

The results here reported concern the behavior in tissue culture of the hypothalamic grey matter which lies close to the wall of the third ventricle of mammals.

Culture of adult nervous tissue as recently reported by Costero and Pomerat (1951, 1952) has been directed toward the cerebral and cerebellar cortex. It was believed of some importance to determine whether the vegetative centers also could be maintained in culture. As a first approach, in order to avoid the cultivation of nuclei too widely different in structure, the small hypothalamic area close to the third ventricle was chosen. Here the main features are the paraventricular and supraoptic nuclei to which several authors have attributed a secretory function.

Materials and Methods

The animals used were three cats (one adult and two about one month old), two adult rabbits (which had been used for skin grafting experiments), two dogs (one adult and one newborn), and an adult guinea pig. During the course of the animal experiments a 5-month human fetus became available and it was thought profitable to run parallel experiments with this material for comparison.

The animals were killed by decapitation without anesthesia except an adult cat and an adult dog which received sodium nembutal. The brain was removed with the usual aseptic precautions. It was split along the middle sagittal line and each hemisphere was laid out with the cut surface up. The area chosen for the culture corresponded to the anterior and lower part of the wall of the third ventricle, more exactly, to the anterior and middle part of the hypothalamus.

According to Le Gros Clark (1936), Rioch (1929), Fulton (1949), Miller (1942), and Greving (1928), the explanted area contains the whole of two nuclei: (1) the *nucleus supraopticus* (so called by Greving and Le Gros Clark but named "nucleus tangentialis" by Rioch, Cajal (1911), Ingram, Hannett and Ranson (1932), and Roussy and Mosinger (1946)) and, (2)

* Fulbright fellow, from the Tissue Culture Laboratory, The University of Texas, Medical Branch, Galveston. This investigation was supported by a research grant (PHS B-364) from the National Institute of Neurological Diseases and Blindness, of the National Institutes of Health, Public Health Service, administered by C. M. Pomerat.

the *nucleus paraventricularis* (Malone (1912), Morgan (1930), and Roussy and Mosinger (1946)), called "nucleus filiformis" by Gurdjian (1927) or "nucleus filiformis principalis" by Rioch (1929) and Ingram, Hannett and Ranson (1932).

These two nuclei are reported by various researchers. Poppi (1930), Scharrer (1933a and b, 1937), Gaupp and Scharrer (1935), Scharrer and Scharrer (1940), Scharrer, Palay, and Nilges (1945), Bargmann and Scharrer (1951), Scharrer and Gaupp (1933), Gaupp (1934), Roussy and Mosinger (1934), and de Oliveira e Silva (1935a and b) to show in their cells such features as peculiar shapes of nuclei, rich vascularization, cytoplasmic vacuoles, droplets and colloid masses which these authors interpret as signs of secretion, although there are other workers such as Collin (1951) who give the same facts a different interpretation.

Around these nuclei, the area removed for cultures contains other cells which form a less sharply defined zone and belong to a part of what some authors call in a general way the "central or periventricular grey matter" (Greving (1928); Roussy and Mosinger (1946)), while others divide it into different nuclei (nucleus ventromedialis, dorsomedialis and posterior—Le Gros Clark (1936) and Rioch (1929)).

In the adult and in young animals three landmarks facilitate identification of the area with some degree of precision so that tissues removed were quite uniform in all experiments: the mammillary body, the optic chiasma, and the anterior commissure. All three are prominently white against a grey background. These three landmarks form three corners of a rectangular area which is completed by imagining the fourth corner at the same height above the mammillary body as the anterior commissure (Fig. 1).

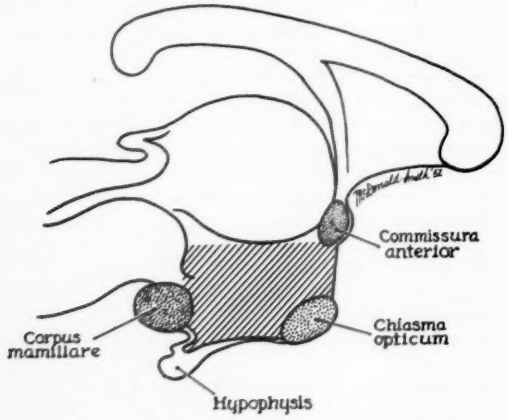

FIG. 1. The area selected for cultures of the hypothalamus of mammals is indicated by parallel lines.

With a sharp knife held horizontally a thin slice of nervous tissue was taken out which contained the grey matter lying very close to the wall of the third ventricle. The material was cut into pieces about 1 mm. square and cultivated in roller tubes with a solid phase consisting of equal parts of cockerel plasma and chick embryo extract and the usual fluid phase composed of 50 per cent human ascitic fluid, 45 per cent Gey's balanced salt solution, 5 per cent chick embryo extract and penicillin 1000 units/ml. There were 30–40 explants in each experiment which were kept in culture approximately 26 days.

In the human fetus and in the newborn dog the area was not outlined so definitely because of the difficulty of recognizing the usual landmarks at this stage. In addition, the tissue was so soft as to prevent a precise cutting. In these two cases, therefore, the area which was cultured corresponded only approximately to the zone used for the experiments with the other animals.

With the appearance of outgrowth from the 5th day of cultivation, cultures were observed in the living state both with ordinary light and with phase contrast microscopy. Vital staining with methylene blue according to the technique suggested by Costero and Pomerat (1951) was systematically done.

Still pictures and phase contrast, time-lapse moving pictures were taken with an 8 mm. objective while still pictures were also obtained with oil immersion using phase contrast optics.

Many of the cultures were fixed and stained with Gomori's chrome alum hematoxylin. The original method was suggested by Gomori (1941) in order to differentiate the cells of the pancreas. Other authors (Bargmann (1949), Bargmann and Scharrer (1951), Scharrer (1951), and Drager (1953)) applied it to histologic sections of brain showing its specificity to demonstrate granules, droplets and colloid masses in the cytoplasm of periventricular and preopticus nuclei which these authors accepted as signs of secretory activity.

Although in our cultures no cytoplasmic granules were found the method proved very useful in revealing many details of the nuclear and cytoplasmic structures and also in clearly and quickly differentiating between the mesenchyme-like cells which always stained very faintly and the parenchymal cells which always took on a more intense color.

For staining the cultures, the method was simplified and the phloxine counterstain was not used. The procedure was to (1) fix in Bouin's fluid for about 2 hours; (2) pass into 70 per cent alcohol and to change alcohol repeatedly until a yellow color was no longer obtained; (3) wash in distilled water; (4) treat for about 1 minute with a solution containing about 0.3 per cent each of potassium permanganate and sulfuric acid; (5) decolorize with a 2–5 per cent solution of sodium bisulfite and then to wash in distilled water; (6) stain in a hematoxylin solution, under microscope control, with equal parts of 1 per cent aqueous solution of hematoxylin and of 3 per cent solution of chromium alum. Add to each 100 ml. of the mixture 2 ml. of 5 per cent solution of potassium dichromate and 2 ml. of 2.5 per cent sulfuric acid. The mixture is ripe after 48 hours and can be used as long as

a film with a metallic luster will continue to form on its surface, and filter before use; (7) differentiate in 1 per cent hydrochloric acid-alcohol for about 1 minute (necessary only when the culture is too deeply stained); (8) wash with tap water until the section is a clear blue, and (9) pass through the alcohols carbolxylol xylol and to mount in balsam.

General Behavior of the Cultures. The cultures did not show signs of activity until the 5th or 6th day when a few cells began to emigrate. Only after a further 3–4 days in culture did the outgrowth begin to be conspicuous and a layer of cells form which kept increasing slowly during the time the cultures were maintained.

Zones of liquefaction in the solid medium were commonly encountered. Often the area of liquefaction did not surround the explant but only a part of its contour. The macrophages frequently tended to migrate to these zones.

In many cultures of cat tissue in which glial cells were sufficiently numerous a definite positioning was found so that there were three zones of cells: the internal one, corresponding to a zone of liquefaction and full of macrophages; a middle zone chiefly occupied by star-like astroglial cells; and an external ring of mesenchyme-like cells. The nerve cells were few and had no definite position, being scattered throughout the outgrowth area. The types of cells commonly observed in these cultures were mesenchyme-like cells, macrophages and parenchymal cells, *i.e.*, astroglia, oligodendroglia and nerve cells.

The best migration of parenchymal cells was observed in the cat and dog cultures. In the rabbit and guinea pig there was a preponderance of mesenchyme-like cells with a comparatively small number of parenchymal cells. In the human fetus there was a strong development of mesenchyme-like cells. This may have been due either to the age or to the inclusion of a different area as a result of the difficulty of identification. The rabbit cultures showed a large and early migration of macrophages which differed from the other species studied where macrophages migrated later and in comparatively small numbers.

These differences in cellular behavior may have been due to an actual species difference, slight differences in the area taken for cultures or in the human fetus to an age difference. The rabbit material was derived from animals which had been used for homografting and for the preparing of an anti-erythrocytic

factor some weeks before they were used for the present study.

Mesenchyme-Like Cells. The mesenchyme-like cells observed in these cultures will be described only briefly in order clearly to differentiate them from parenchymal cells. They were the earliest to migrate and reached the greatest distances. When a zone of liquefaction was found most mesenchyme-like cells were beyond it while the liquefaction zone itself contained chiefly macrophages. The shape of the mesenchyme-like cells varied considerably, some were stellate while others were spindle-shaped. Processes arising from these cells usually had a broad base which had the appearance of a cytoplasmic extension in sharp contrast to parenchymal cells where the processes were filamentous over their entire length and did not have any expansion at their bases. The cells were flat with thin transparent cytoplasm containing granules around the nucleus which were refractive in the living state. Their numerous mitochondria were chiefly filamentous. The nuclei were always large, round to oval, thin and very transparent. They contained two or more irregularly-shaped, small nucleoli. The karyoplasm between these nucleoli was homogeneous in the living cell but became indistinctly granular upon fixation. These mesenchyme-like cells were presumed to come from the walls of blood vessels contained in the explant. The rich vascularity of the paraventricular nucleus has been described by Scharrer and Gaupp (1933). The outgrowth of these elements is represented in figures 2, 3 and 7.

Macrophages. It is not necessary to describe macrophages in detail because they are easily recognizable and not different from those ordinarily encountered in tissue cultures. They were more numerous in the outgrowth from rabbit tissue and more frequent in the zones of liquefaction. Possibly this was due to their motility and consequent ease of motion in the liquefied area. Several living macrophages filled with ingested material are shown in figure 3.

Parenchymatous Cells. Generally speaking, these elements are recognizable by being smaller than the mesenchyme-like cells and because they do not tend to spread out. In addition, they are less transparent, more refractive in the living state and more densely stained after fixation. They do not tend to mix with the mesenchyme-like cells and migrate at different levels

from them in the plasma clot. Parenchymal cells can be found in any part of the culture although they show a certain preference for the margins of the liquefaction zone.

Astroglia. The largest numbers of parenchymal cells are glial, the majority of which are readily identified as astroglia. When they are typical their features are, (1) a nucleus which is very frequently eccentric, even protruding from the cell. It is fairly clear, usually round or oval and with only one nucleolus; (2) a globose perikaryon which is rather opaque and brilliant when observed in the living state with phase contrast optics but dark and dense when stained with Gomori's hematoxylin; (3) bipolarity or with a great number of processes which come directly from the surface of the perikaryon with tapering cones of emergence. These are often very numerous, relatively straight, radiating in all directions and occur sometimes in tufts and without protuberances on their surface; (4) very numerous delicate membranes of two major types. The first is a wide membrane that completely surrounds the perikaryon and extends between the processes so as to occupy the intermediate spaces like an interdigital membrane. The outer margin of this membrane may reach to the very tips of the processes and is frequently difficult to see even with phase optics. In moving pictures the membranes show a slow undulating movement like a bedsheet exposed to a breeze. The second membrane shape, which is possibly a variant of the first, is sometimes seen along the course of an individual process, especially at some distance from the perikaryon. It can hardly be seen in still pictures because of its thinness, but its movement, a kind of spiral undulation around an axis formed by the process, is frequently observed in moving picture records.

In aggregates astroglial processes intermingle and give origin to an extremely thick meshwork, not very different from and perhaps even more complicated than what is known from sections of nervous tissue. Figures 4 and 5 show particularly well developed networks made up of cells which are probably of the

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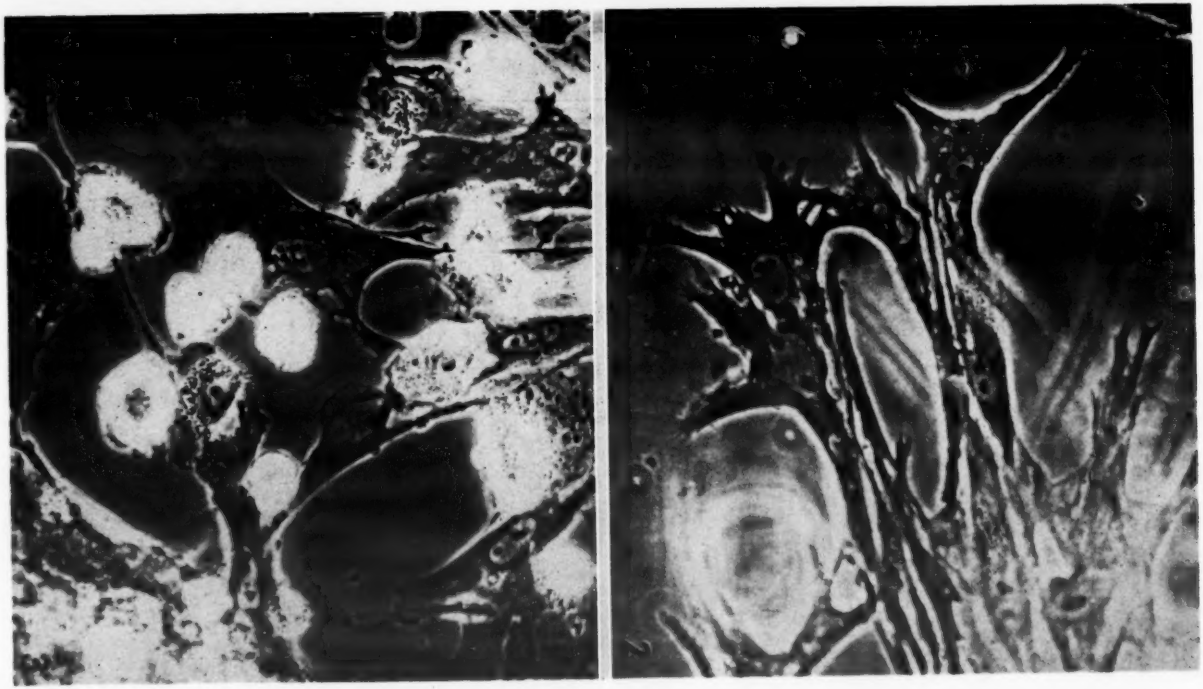
FIGS. 2 AND 3. Mesenchyme-like cells from a 9-day culture of adult cat hypothalamus (fig. 2) and from a 6-day culture of adult rabbit hypothalamus (fig. 3). The bright spherical objects in figure 3 are macrophages. Living preparations photographed with dark phase contrast microscopy. Approximately 400X.



type generally known as protoplasmic astrocytes. The processes and the membranes connected with them compose a net containing moving macrophages which are seen as highly refractive spots in figure 4. Cinematographic records suggest that this movement takes place with some difficulty and that the macrophages keep changing their shape, adapting themselves to the meshes of the net.

Oligodendroglia. These consist of small cells with a few processes. They are frequently seen in small groups or in clusters. The cell body is highly refractile in the living state with processes of varying lengths (see figures 4 and 6). Although it was extremely difficult to identify these elements, it should be noted that they have a certain similarity to the cells which Pomerat (1951) believes to be oligodendroglia. However, no pulsatile movement in cells with these features was observed in this series of cultures.

Neurons. As Costero and Pomerat (1951, 1952) pointed out, the identification of nerve cells is extremely difficult due to their similarity to neuroglial elements. Nonetheless, with the use of the criteria which follow, some neurons were found in tissues from all species studied, but the cat and dog material proved to be particularly fruitful.

(1) Shape of the cells. Nerve cells were usually bipolar or with a few processes which were occasionally branched. However, the bipolar cells which were sometimes found in a zone of liquefaction, with one end attached to the explant and the other to the clot, were not necessarily neurons. The cell shape here depended not so much on an inherent cell characteristic as on mechanical tension. Sometimes two or three cells were in line and united by a process which seemed to belong to both. The processes sometimes had a thorny appearance and emerged from small cones or from the angles of the cell body. When the processes showed an angular course and beadings, this could be considered a nearly certain proof that they were neurons. Fig-

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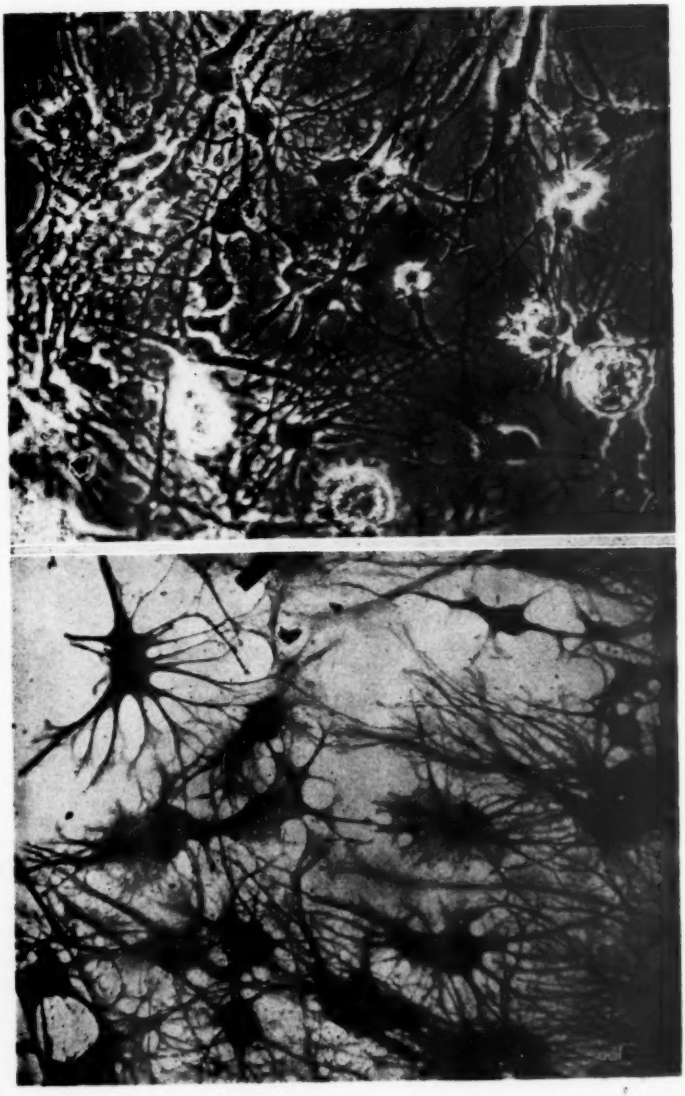
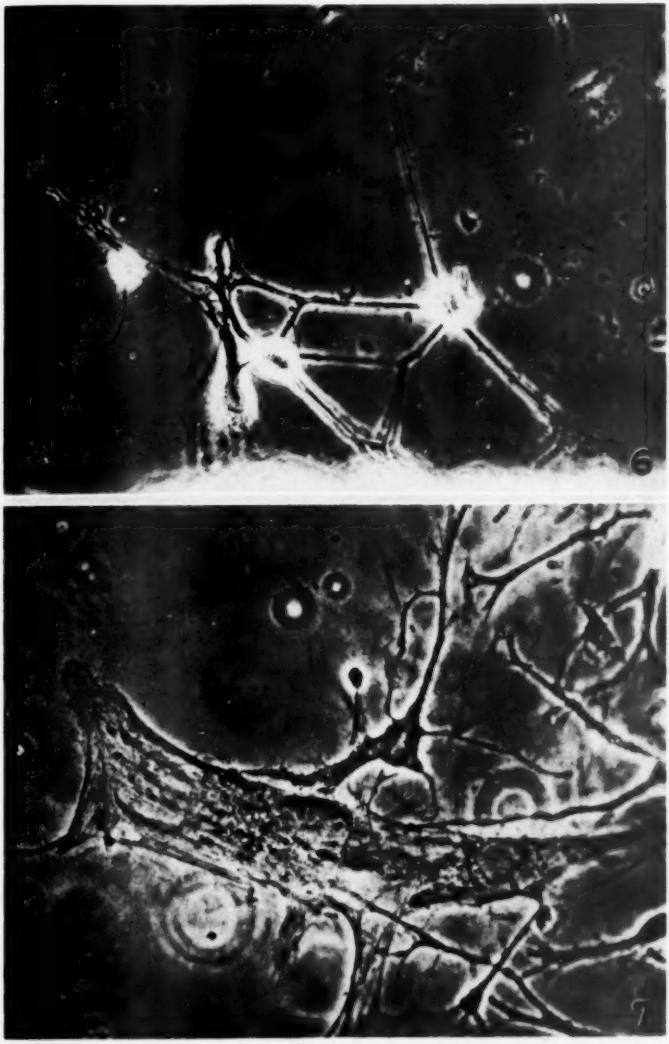
FIGS. 4 AND 5. Networks of glia composed primarily of the type generally described as protoplasmic astrocytes from adult cat hypothalamus. Figure 4 which represents living elements as seen with dark phase contrast microscopy was derived from a 9-day culture. The arrow is directed at a cell believed to be of the oligodendroglial type. The cells in figure 5 from a 13-day culture were stained with Gomori's hematoxylin. Approximately 400X.

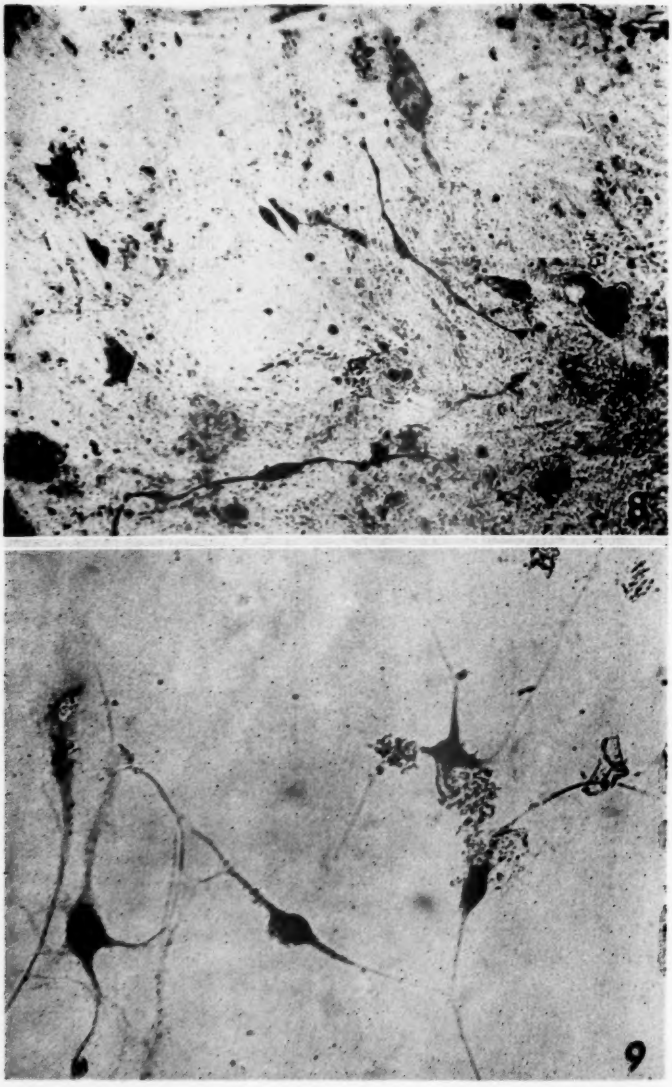


ure 7 from a cat culture shows a living element with all the features of a nerve cell, including a large round nucleus with one nucleolus, three fine processes and one ribbon-like process. (2) Structure of the cell. Neurons have a very opaque cytoplasm which is full of granules and thus appears strongly refractive in the living cell. This cytoplasm stains more darkly than that of other cell types present. (3) Staining with methylene blue. This method is very valuable, but it should be used with great care. Neurons rapidly accumulate a blue color in their cytoplasm from a medium containing methylene blue at 1:50,000. Experience is necessary to distinguish a really specific reaction from the progressive color reaction which other types of cells exhibit as irreversible injury proceeds. Macrophages may take some methylene blue in their large granules but these cells are easily recognized. Glia may show small blue granules in the perinuclear area, but they are less deeply stained than those in the nerve cells in the early period of observation. All cells, initially, may take up a certain amount of dye in cytoplasmic granules but in the next thirty minutes or so the stain becomes paler except in some elements where it becomes more and more intense and acquires the typical features of a specific stain, that is, deep staining of the cytoplasm and processes while the nucleus remains still clear with the exception of the nucleolus which takes the stain. With time, beads accumulate in the processes. Figure 8 shows two bipolar cells specifically tagged with methylene blue with characteristic bending and beading along the course of their processes. Figure 9 shows a neuron with a typical nucleus and four processes coming from cones. Glial elements in the close vicinity did not accumulate the dye. (4) Movement. Neurons are characterized by a relative lack of motion when observed in moving pictures. Nerve cells were found in all parts of the cultures. Sometimes they were at the outer margin of the mesenchymatous outgrowth. In these cases it may be that they were carried out passively by the mesenchymal cells as suggested by Costero and Pomerat (1951).

Intracytoplasmic colloid granules or masses which in histologic sections stain very deep dark blue and which are described by various authors to be the result of secretory activity could not be found in these cultures in spite of observation with the modified Gomori method. It may be that this was because the



FIGS 6 AND 7 were made from 9-day living cultures of adult cat hypothalamus with dark phase contrast optics. All the elements in figure 6 are believed to be oligodendroglia while in figure 7 a typical neuron is seen adjacent to a well extended cell of the mesenchymatous type. Approximately 400X.



FIGS. 8 AND 9. Neurons from adult cat hypothalamus as revealed with the use of methylene blue at 1:50,000 with ordinary light microscopy. Figure 8 was obtained from a 7-day culture.

In figure 9 which was made from a 13-day culture two bipolar and a multipolar neuron are shown with two glial elements which contained some blue granules in the juxtanuclear area. Approximately 400X.

number of nerve cells emigrating was so small that there was only a very slight probability of their containing any secretory cells or that their products were rapidly expelled into the culture medium. Further investigation will be necessary to clarify these problems.

Summary

Explants of hypothalamic nuclei from several species of mammals including a five-month human fetus were cultured in roller tubes and studied during a three-week period with phase still and cinematographic technique, as well as with methylene blue and Gomori's hematoxylin method. Mesenchyme-like cells, astroglia and neurons were observed in cultures of all species examined. No evidence was found for the production of neurosecretion *in vitro*.

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STUDIES ON TOXIC PLANTS FOR ANTIBACTERIAL EFFECTS II*

HENRY M. BURLAGE, SUSAN M. GIBSON, GEORGE F. MCKENNA, AND ALFRED TAYLOR

Although numerous studies on the production of antibiotic substances by higher plants have been carried out, the field still offers innumerable possibilities for research. A number of workers have experienced notable success in detecting antibacterial agents in higher plants (Baron, 1950; Karel, 1951), a few of which have been isolated and studied in detail.

For the past three years it has been the purpose of these studies to screen extracts of various plants for antagonistic action upon bacteria, virus, and tumors (Taylor, *et al.*, 1951; Taylor, *et al.*, 1952). In addition, the toxicity to experimental animals of many of these extracts has also been investigated. In a previous report (Burlage, *et al.*, 1952), the methods and results of the antibacterial studies were described. The plants investigated in this program are, in general, those indigenous to Central Texas which have been reported as toxic to animals and/or to have medicinal action.

Methods

In the primary testing of each plant, all parts are extracted together. If a plant has showed activity in previous testing, the various parts are subjected to extraction separately in order to determine the plant parts with the highest concentration of active principle(s).

Seven solvents are being used at the present time: distilled water at pH 6, pH 7, and pH 8, alcohol, acetone, chloroform, and petroleum ether. From each plant or plant part four water extracts are prepared, one each at pH 6, pH 7, and pH 8, and a water extract (pH 7) in which the solvent is boiled with the plant material for fifteen minutes. Alcohol, acetone, chloroform, and petroleum ether are used successively on the same sample of plant material for selective extractions. In addition, an acetone extract is prepared from a fresh plant sample.

In most cases the sample of plant material is fresh or preserved by freezing, but sometimes the plants are dried under reduced pressure for storage until they can be utilized.

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* From the Pharmaceutical Foundation of the College of Pharmacy and the Biochemical Institute of The University of Texas and the Clayton Foundation for Research, Austin.

In the preparation of extracts, the plant material is finely ground with the solvent in a Waring Blendor and then stored in the refrigerator. After the desired extraction time, usually one week, the plant material is removed by filtration. The filtrates of the water extractions are freeze-dried, and the resulting dry extracts made up to a known concentration in triple distilled water. The organic solvents are evaporated under reduced pressure, and triple distilled water is added to the residues. This water-soluble portion of the organic solvent extract is freeze-dried and made up to a known concentration.

All extracts are sterilized by filtration through bacterial filters and tested for sterility in semi-solid brain heart infusion.

During the process of extraction and subsequent storage, every effort is made to retain all active constituents of the original material. The precautions taken in view of this goal are: maintenance of temperatures as low as practicable, colored glassware to reduce photochemical effects of light, aseptic technique, and lyophilization of extracts before prolonged storage.

TABLE 1

Plants whose extracts showed no antibacterial action

Ambrosia aptera DC	Papaya sp. (leaves and roots separately)
Astragalus Earlei Greene[1]	*Parkinsonia aculeata* L
Astragalus Wootoni Sheldon	*Parthenium Hysterophorous* L.
Cannabis sativa L	*Phoradendron flavescens* (Pursh) *Nutt.*[1,2]
Cichorium intybus L.[1]	*Rhus glabra* L.
Curcurbita foetidissima H.B.K.	*Salvia farinacea* Berth
Datura sp.	*Salvia Greggii* A. Gray
Gossypium sp.	*Sophora secundiflora* (Ort.) Log[1]
Liatris punctata Hook.	*Tecoma radicans* L.[1]
Melia Azedarach L.	*Verbena bipinnatifida* Nutt.
Nicotiana glauca Graham	*Vitis candicans* Engelm.[1]
Mexican Plants	
Acocotillo [1,3]	
Acolypha phleoides Cav. [1,2,3]	
Liucpia plata	

[1]Positive results shown in Table 2.
[2]Positive results shown in Table 3.
[3]Dried drug obtained from and identified by Mexican herbalist.

TABLE 2

Plants and their extracts showing slight antibacterial activity (Zones less than 5 mm.)

Plant	Plant part and solvent	Organism
Acocotillo[1]	Seed Acetone	Pseudomonas aeruginosa Shigella dysenteriae
Acolypha phleoides Cav.[1]	Dried whole plant H_2O pH 7.0	Salmonella typhosa

[1]Dried drug obtained from and identified by Mexican herbalist.

Plant	Plant part and solvent	Organism
Astragalus Earlei Greene	Stem, leaves and fruit H_2O pH 6.0	Staphylococcus aureus
	Stem, leaves and fruit H_2O pH 7.0 H_2O pH 7.0 boiled	
Cichorium intybus L.	Whole plant Acetone	Staphylococcus aureus
Phoradendron flavescens (Pursh) Nutt.	Leaves, stem and fruit H_2O pH 7.0	Escherichia coli
	Acetone	Neisseria catarrhalis Pseudomonas aeruginosa Shigella dysenteria
	H_2O pH 7.0	Salmonella paratyphi A Salmonella paratyphi B Salmonella typhosa Shigella dysenteria
	Fruit Absolute alcohol	Salmonella typhosa
	Acetone	Escherichia coli
	H_2O pH 7.0 boiled	Staphylococcus aureus Salmonella typhosa
Rhus toxicondendron	½ leaves and ½ stems H_2O pH 6.99 boiled	Pseudomonas aeruginosa
var. *radicans* (L) Torr.	stems, green H_2O pH 6.6	Salmonella paratyphi B
	Leaves, blooms, and stems, green	Bacillus subtilis
Sophora secundiflora (Ort.) Log.	Fruit and leaves Acetone Absolute alcohol	Staphylococcus aureus
Tecoma radicans L.	Leaves, stems, roots Absolute alcohol	Salmonella paratyphi A
Vitis candicans Englem.	Leaves and stems Acetone	Staphylococcus aureus Salmonella paratyphi A
	H_2O pH 8.0	Salmonella paratyphi A

TABLE 3

Plants and extracts showing antibacterial activity against more than one test organism

Plant	Plant part pH of solvent	Staphylococcus aureus	Escherichia coli	Proteus Vulgaris	Salmonella paratyphi A	Salmonella paratyphi B	Salmonella typhosa	Neisseria catarrhalis	Pseudomonas aeruginosa	Shigella dysenteria	Bacillus subtilis
Acolypha phleoides Cav.[1]	Ground plant parts Acetone	2 mm	..	..	..	..	..	..	..	5 mm	..
Phoradendron flavescens (Pursh) Nutt.	Leaves, stems and fruit Abs. alcohol	..	..	..	..	8 mm	..	..	..	..	..
	Leaves, stems and fruit H_2O pH 6.6	..	5 mm	..	..	..	..	..	..	..	..
	Fruit H_2O pH 7.0	..	..	..	..	5 mm	..	..	..	..	1 mm
	(bottom layer)	..	..	..	..	..	..	..	..	3 mm	..
	(2 distinct top layer)	9 mm	5 mm	4 mm	11 mm	7 mm	11 mm	..	..	10 mm	5 mm

	Fruit H_2O pH 8.0	..	..	..	..	5 mm	..	..	..	..	..
Rhus toxicodendron var. *radicans*	½ leaves and ½ stems Acetone	1 mm	..	..	..	2 mm	1 mm	2 mm	5 mm	..	2 mm
(L.) Torr.	½ leaves and ½ stems H_2O pH 7.5	.. ..	1 mm ..	.. ..	.. ..	.. ..	.. ..	.. ..	6 mm 6 mm 2 mm[2]	.. ..	.. ..
	Leaves, green stems, and blooms Abs. alcohol	1 mm	5 mm	..	..	..	..	..	2 mm	4 mm	2 mm
	Leaves, green stems, and buds Acetone	..	..	..	..	6 mm	3 mm	2 mm	2 mm	2 mm	4 mm

[1]Dried drug obtained from and identified by Mexican herbalist.
[2]Cup method.

Although a number of plate methods for measuring inhibitory action have been employed in the past, the filter paper disc method seems most suited to this program. Plates are prepared with nutrient agar, pH 7.2±.1, which has been seeded in bulk with the stock organism (1 ml. of a 20 hour broth culture per 25 ml. of fluid nutrient agar). Under aseptic conditions discs of highly absorbent filter paper, ½ inch in diameter, are transferred to the surface of the agar, and 0.05 ml. samples of the test solutions are pipetted on the discs. After the plates have incubated for 20 hours, the zones of inhibition are measured from the disc to the edge of the zone.

The organisms employed in the testing program are: *Micrococcus pyogenes* var. *aureus* FDA 209, *Escherichia coli* ATCC 9637, *Proteus vulgaris* ATCC 9623, *Bacillus subtilis* ATCC 6633, *Salmonella paratyphi* ATCC 9444 (*Salmonella paratyphi* A), *Salmonella schottmuelleri* ATCC 10258 (*Salmonella paratyphi* B), *Salmonella typhosa* ATCC 6229, *Neisseria catarrhalis* ATCC 7900, *Pseudomonas aeruginosa* ATCC 10197, and *Shigella dysenteriae* ATCC 9665.

Results

Tables 1–4 show the results of the most recent studies conducted.

Discussion. When working with plants as they occur in nature, certain problems arise which do not confront the investigator of microorganisms. The greatest of these problems is the uncontrollable growth conditions. It has been noted by other workers that seasonal variation has a profound influence on the presence of antibacterials in plants (Pratt, *et al.*, 1951; Taylor, *et al.*, 1952). It is reasonable to assume also that soil, temperature, and moisture conditions exhibit an influence. Plant organs give very different results (Lucas, *et al.*, 1951). Some additional work with selected plants is being undertaken.

Acetone extracts are not consistent as can be seen from the tables. At present, a suitable explanation is not forthcoming. Time and additional work may provide an answer.

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STUDIES ON GASTRIC MUCIN

III. The Effect of Gastric Mucin on the Antibacterial Action of Normal Blood*

Frank B. Engley, Jr., and Martha Jordan Scott

A review of the literature on Mucin as a Resistance-Lowering Substance (Olitzki, 1948) has summarized the many theories relative to the mode of action of mucin *in vivo*. It has been proposed that it enhances the virulence of the organism, acts as a culture medium supplying nutrients and growth factors to the organism, lowers the resistance of the animal to infection by the toxicity of substances in the mucin, destroys complement thus preventing lysis of the cells, coats the cells with a covering protective against the host defenses, inhibits antibody absorption by the organism, inhibits the appearance of leukocytes, inhibits phagocytosis and/or intracellular digestion of bacteria by fixed and mobile cells, increases the permeability of the barrier between the abdominal cavity and the blood stream, and neutralizes or inhibits the bactericidal action of blood and body fluids. Further evidence is presented here for the latter theory.

The antibacterial or antimicrobial action of whole blood and its components has been the study of numerous workers and is summarized in standard texts of bacteriology and immunology such as Topley and Wilson, 1946. In brief, the leukocytes besides being phagocytic have been shown to produce leukins and the platelets form plakins both of which have antibacterial activity. The red cells apparently do not have antibacterial activity in the form and concentration in which they are present in the blood stream. The fluid portion of the blood contains the heat labile complement which is active with antibody in aiding phagocytosis as well as lysis of susceptible bacterial cells. Also present in the serum or plasma are heat stable antibacterial substances which can act without complement. These heat stable

* From the Department of Bacteriology and Parasitology, The University of Texas, Medical Branch, Galveston. This investigation was supported by a contract AF 41(607)-41 with the USAF School of Aviation Medicine, Randolph Field, Texas.

substances have been termed β (beta) lysins and are not well defined or completely understood. In studying the antibacterial action of whole blood then, we find that there is a series of complex mechanisms of cellular and humoral and cellular-humoral combinations involved in the protection of the host from invading organisms.

Preliminary and survey reports by the author (Engley, 1950, 1953a, 1953b, 1953c) have been made on some of these studies. In studies with *Shigella dysenteriae* as an indicator organism in infections in mice data revealed different levels of activity from various lots of gastric mucin and from different strains of *S. dysenteriae* in the same lot of mucin. Addition of the test organisms is relatively small numbers (10^1–10^5 per ml.) to fresh blood, plasma, serum and peritoneal washings resulted in their destruction. Heated serum showed residual activity against the organisms indicating that complement was not active in the process, and that the activity is probably associated with the Beta lysins of the blood and plasma. Addition of gastric mucin resulted in growth of the organism in the body fluids. The antibacterial activity was found in the blood and plasma or serum of a number of species tested: mice, rabbits, cows, sheep, and humans. Purified fractions of both bovine and human plasma, alone and recombined, showed no activity, suggesting that the active material was destroyed in the procedures involved in fractionation and storage. Preliminary fractionation of gastric mucin revealed that the inhibiting substance for the antibacterial action of plasma on *S. dysenteriae in vitro* and *in vivo* was in a water insoluble residue. Comparative studies with gastric mucin and other substances such as gums, starch and sugars showed good correlation as to their inhibiting or neutralizing ability *in vivo* and *in vitro*. However, none of the substances tested was as active as the gastric mucin. Those which showed some activity were difficult to prepare and their high viscosity made them hard to work with when using pipettes and syringes.

These findings showing that normal serum, plasma and blood had antibacterial activity apparently unassociated with complement and antibody appeared of interest in clinical blood culture studies. Of equal interest with the antibacterial activity was its neutralization by gastric mucin in the test tube and in the body. It was felt that these findings warranted further studies on the

antibacterial action of normal blood and its neutralization by gastric mucin.

Results are reported here of further studies in which *Escherichia coli* was employed as a test organism in investigations into some qualitative and quantitative aspects of the antibacterial action of blood. Several species of animals were utilized in determining the spectrum of the antibacterial action of blood to determine the types of bacteria susceptible to the blood action. Some quantitative relationships in the neutralization of the antibacterial action of blood by gastric mucin have been investigated.

Experimental

Exploratory studies on normal rabbit blood were made to determine the spectrum of organisms susceptible to the action of fresh blood. The original group of organisms included *Sarcina lutea, Bacillus subtilis, Aerobacter aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Escherichia coli, Alcaligenes fecalis, Paracolobactrum aerogenoides* and *Klebsiella pneumoniae*. The tests were performed as follows. One ml. aliquots of fresh defibrinated blood were placed in Wasserman tubes and 10^3 or 10^6 viable cells were added in a 0.1 ml. inoculum of the test organism. After

TABLE 1

The Antibacterial Action of Rabbit Blood for Various Organisms

Organism	Number	Sampling Time 1 hour	3 hours	6 hours	12 hours	24 hours
Sarcina lutea	10^3	—	—	—	—	—
	10^6	—	—	—	—	1+
Bacillus subtilis	10^3	—	—	—	—	—
	10^6	—	—	—	—	—
Aerobacter aerogenes ..	10^3	—	—	1+	4+	4+
	10^6	4+	4+	4+	4+	4+
Pseudomonas aeruginosa	10^3	—	—	1+	3+	4+
	10^6	4+	4+	4+	4+	4+
Proteus vulgaris	10^3	+	+	4+	4+	4+
	10^6	4+	4+	4+	4+	4+
Staphylococcus aureus..	10^3	—	—	—	3+	4+
	10^6	3+	2+	4+	4+	4+
Escherichia coli	10^3	—	—	—	—	—
	10^6	2+	3+	1+	4+	4+
Alcaligenes fecalis	10^3	—	—	1+	3+	4+
	10^6	3+	3+	4+	4+	4+
Paracolobactrum aerogenoides	10^3	—	—	2+	4+	4+
	10^6	4+	4+	4+	4+	4+
Klebsiella pneumoniae..	10^3	—	—	—	—	—
	10^6	4+	4+	4+	4+	4+

—No growth.
+Amount of growth.

intervals of 1, 3, 6, 12 and 24 hours incubation at 37° C., a standard loop of the mixture was streaked on a segment of nutrient agar in a petri plate. These plates were incubated for 24–48 hours at 37° C. to determine whether viable bacteria were present in the blood. Quantitative relationships were established by rating the resultant growth by the (+) system. (— no growth, 1+ few colonies, 2+ numerous colonies, 3+ confluent growth and 4+ heavy confluent growth.)

The results of a typical experiment are shown in table 1. These findings are similar to a large series of rabbit bloods tested in this manner. *Sarcina lutea* and *Bacillus subtilis* appeared most susceptible while *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Alcaligenes fecalis*, *Aerobacter aerogenes* and the paracolon organism were among the more resistant of the group tested. Considerable difference in results was noted depending on whether 10^3 or 10^6 organisms were used as the inoculum. The larger 10^6 (one million) cell concentration was considered somewhat large for a test inoculum since the majority of strains appeared resistant to any blood action. The 10^3 (one thousand) cell inoculum was chosen for much of the subsequent spectrum testing since it provided a fairly sensitive test and was more in line with the maximum numbers of cells which the blood might contain in bacteremias and septicemias.

Similar tests with sheep blood and human blood at the 10^3 level of organisms revealed that only *Pseudomonas aeruginosa* and *Staphylococcus aureus* were at all resistant to the antibacterial action of the sheep blood (Table 2). Results of testing the

TABLE 2

*The Antibacterial Activity of Blood from Various Species**

Organism	Species		
	Rabbit	Sheep	Human
Escherichia coli	—	—	—
Bacillus subtilis	—	—	—
Aerobacter aerogenes	4+	—	—
Klebsiella pneumoniae	—	—	—
Proteus vulgaris	4+	—	—
Staphylococcus aureus	4+	4+	4+
Pseudomonas aeruginosa	4+	4+	—
Salmonella typhosa	x	x	—
Alcaligenes fecalis	4+	—	—
Sarcina lutea	—	—	—

*10^3 organisms inoculated per ml. Samples taken after 24 hours at 37° C.
—No growth.
+Amount of growth.
x Not tested.

same group of organisms in human blood revealed activity against all of the test organisms except *S. aureus*. These gradually increased in number in the blood during the 24-hour test period (Table 2).

A summary of the findings with a larger series of representative organisms in human blood samples (Table 3) suggest that

TABLE 3

The Effect of Mucin on the Spectrum of Antibacterial Activity of Human Blood

Enterobacteriaceae	Blood	Blood plus Mucin	Bacillaceae	Blood	Blood plus Mucin
Escherichia coli	+	—	Bacillus subtilis	+	—
Aerobacter aerogenes	+—	—	Bacillus globigii	+	—
Paraclobactrum aerogenoides	+—	—	Bacillus anthracis	+—	—
Serratia marcescens	+	—	Clostridium perfringens	+—	—
Proteus vulgaris	+	—	*Micrococcaceae*		
Proteus morganii	+	—	Micrococcus spp		
Salmonella typhosa	+	—	(Staph.) aureus	+—	—
Salmonella paratyphi A	+	—	albus	+—	—
Salmonella schottmuelleri	+	—	citreus	+	—
Salmonella typhimurium	+	—	Sarcina lutea	+	—
Klebsiella pneumoniae	+—	—	*Lactobacteriaceae*		
Shigella dysenteriae	+	—	*Streptococcae*		
Shigella flexneri	+	—	Streptococcus pyogenes	+—	—
Shigella sonnei	+	—	Streptococcus viridans	+	—
Pseudomonadaceae			Diplococcus pneumoniae	+	—
Pseudomonas aeruginosa	+—	—	*Neisseriaceae*		
Achromobacteriaceae			Neisseria catarrhalis	+	—
Alcaligenes fecalis	+	—	Neisseria meningitidis	+	—
Parvobacteriaceae			Neisseria gonorrhoeae	+	—
Pasteurella multocida	+	—	*Corynebacteriaceae*		
Brucella abortus	+	—	Corynebacterium diphtheriae	+	—
Brucella suis	+	—	Corynebacterium xerose	+	—
Hemophilus influenzae	+	—	Corynebacterium pseudo-diphtheriticum	+	—
Hemophilus parainfluenzae	+	—			

+Susceptible to blood activity.
+—Susceptibility varies.
—Not susceptible.

a wide spectrum of bacteria is susceptible to inhibition or destruction by normal blood when added in small numbers (10^3) under the conditions of test. Addition of larger numbers of organisms resulted in some resistance on the part of *Aerobacter aerogenes, Paracolobactrum aerogenoides, Klebsiella pneumoniae, Pseudodomonas aeruginosa, Bacillus anthracis, Clostridium*

perfringens, *Staphylococcus aureus* and *albus* and *Streptococcus pyogenes*.

A brief comparison of levels of activity of sheep, rabbit, and human bloods (Table 2) suggest that different species of animals may have inhibiting levels of a different order against the same test organism and may show a slightly different bacterial spectrum of activity. Also individual rabbits and humans have shown different levels of activity for the same organism and have a slightly different spectrum of activity.

Some qualitative and quantitative studies on the antibacterial activity of human blood were made using *Escherichia coli* as the test organism. Table 4 outlines some typical growth patterns

TABLE 4

*The Effect of Numbers of Organisms on the Antibacterial Activity of Human Blood**

No. of organisms	Sampling Time 3 hours	6 hours	12 hours	24 hours
10^8	4+	4+	4+	4+
10^7	1+	3+	4+	4+
10^6	1+	3+	4+	4+
10^5	1+	3+	3+	4+
10^4	—	1+	3+	3+
10^3	—	—	—	—
10^2	—	—	—	—
10^1	—	—	—	—

**E. coli* at 37° C.
—No growth.
+Amount of growth.

resulting when varying numbers of the test organism were added to 1 ml. samples of human blood, incubated at 37° C. and sampled with standard loop onto segments of nutrient agar plates at varying lengths of time. With 10^1–10^3 organisms, complete inhibition of growth occurred but with increasing numbers at the 10^4 level the organism grew out slowly so that with 10^5–10^8 cell inoculation, heavy growth occurred at the early hours of sampling. Some other samples of blood tested showed activity against *E. coli* up to the 10^6 level and a few only at the 10^2 level.

The effects of anticoagulants and defibrination on the antibacterial activity of human blood were tested, using *E. coli* as the test organism (Table 5) at the 10^3 and 10^6 level in this particular sample of blood. Previous reports by Allgower (1949)

suggested that citrate was toxic for phagocytic activity while another report revealed that anticoagulants such as heparin might reduce complement activity (Lambert, 1952). It was of interest to determine whether the various methods of preparation had any effect on the antibacterial activity of the blood. Heparinized and defibrinated (by shaking) bloods appeared to be slightly more active. Included in this study was a test of the serum and cellular components from the defibrinated blood for activity against *E. coli*. Slight inhibition of the organism was noted when the packed blood cells were added to an equal volume of nutrient broth and the test organism added at 10^3 but not at 10^6 cells per ml. This may be accounted for partly by the carry over of some serum constituents and slight activity by the leukocytes themselves.

TABLE 5

*Antibacterial Action of Human Blood and Blood Components Prepared by Different Procedures**

Types of preparation	Number of cells	Sampling Time 0 hour	3 hours	6 hours	12 hours	24 hours
Heparin	10^3	—	—	—	—	—
	10^6	4+	1+	+	—	—
Oxalate	10^3	—	—	—	—	—
	10^6	4+	1+	4+	4+	4+
Citrate	10^3	—	—	—	—	—
	10^6	4+	4+	2+	4+	4+
Defibrination	10^3	—	—	—	—	—
	10^6	4+	3+	1+	—	—
RBC (Cellular Compenents)	10^3	—	—	—	1+	2+
	10^6	4+	4+	4+	4+	4+
Serum	10^3	—	—	—	—	—
	10^6	4+	4+	2+	1+	—

*Test organism *E. coli* at 10^3 and 10^6 cells per ml. of blood at 37° C. Samples for viability taken at stated intervals. Amount of growth from standard loop on streaked plate given by + system.
—No growth.
+Amount of growth.

It was demonstrated previously that *S. dysenteriae* was not susceptible to the action of the cellular components when tested at a 10^5 level of organisms (Engley, 1950). This indicates the low level of activity of the cellular components in the absence of serum or plasma. The major activity against the bacteria resides in the serum or plasma. Other studies (cited above) showed that the heated serum or plasma still retained antibacterial ac-

tivity revealing that complement was not a major factor in the antibacterial action under test. When testing whole blood for activity against the bacteria it would be difficult to separate the action of cellular components, complement-antibody mechanisms and heat stable antibacterial factors. The antibacterial action is probably cumulative in whole blood, being the sum total of the various mechanisms cited.

The effect of dilution on the antibacterial action of human blood is demonstrated in table 6. In this experiment whole de-

TABLE 6

*The Effect of Dilutions on the Antibacterial Action of Human Blood**

Blood dilution	Number of of cells	Sampling Time 0 hour	6 hours	24 hours	48 hours
1:2	10^3	—	—	—	—
	10^6	—	—	—	—
1:4	10^3	—	—	—	—
	10^6	3+	—	—	—
1:8	10^3	1+	—	—	—
	10^6	3+	—	—	—
1:16	10^3	3+	—	—	—
	10^6	4+	—	—	—
1:32	10^3	3+	4+	4+	4+
	10^6	4+	3+	3+	3+
1:64	10^3	3+	4+	4+	4+
	10^6	4+	4+	4+	4+

*Test organism *E. coli* at 37° C.
—No growth.
+Amount of growth.

fibrinated blood was diluted by the 2-fold serial dilution method using nutrient broth as diluent. The results with this particular blood sample revealed that it was able to inhibit *E. coli* at 10^3 and 10^6 concentrations per ml. when diluted to 1:16. Initial counts at the time of inoculation of the organism into the blood-broth showed growth at low dilutions only which was destroyed by the next test period. Similar investigations with numerous blood samples indicated a range in activity from none when diluted 1:2 to complete inhibition of 10^3 cells at a 1:20 dilution, with most samples active against 10^3 *E. coli* cells at 1:8 and 1:16.

A comparison of fresh and refrigerated human blood was made to determine the effect of age on the antibacterial activity of the blood. Table 7 shows the spectrum of action of a freshly drawn sample of blood and also reveals the loss in activity after

a one-month storage period at 4° C. Several organisms were still susceptible to blood action at that time. (*B. subtilis*, *Paracalobactrum* and *Sarcina lutea*) but the majority of the eleven test organisms grew out in the whole blood within twelve hours of incubation at 37° C.

TABLE 7

*Effect of Ageing on the Antibacterial Activity of Human Blood**

Organism	Age of blood	
	Fresh	Stored 1 month at 4° C.
Escherichia coli	—	4+
Bacillus subtilis	—	—
Aerobacter aerogenes	—	4+
Klebsiella pneumoniae	—	4+
Proteus vulgaris	—	4+
Pseudomonas aeruginosa	—	4+
Paracolobactrum aerogenoides	—	—
Staphylococcus aureus	4+	4+
Salmonella typhosa	—	4+
Alcaligenes fecalis	—	4+
Sarcina lutea	—	—

*10^3 organisms inoculated per ml. Samples taken after 24 hours at 37° C.
—No growth.
+Amount of growth.

Similar studies with *E. coli* as the test organism were made with blood stored at three different temperature levels (37° C., 25° C., and 4° C.) to determine the activity after storage at simulated body, room, and refrigerator temperatures. Data indicate loss in activity within a week at body temperature, within two weeks at room temperature and within a month at refrigerator temperature (Table 8).

TABLE 8

*Effect of Storage Temperature on Antibacterial Activity of Human Blood**

Time in days	Storage Temperature		
	37° C.	25° C.	4° C.
1	+	+	+
2	+	+	+
4	+	+	+
7	±	+	+
14	—	±	+
21	—	—	+
28	—	—	±
35	—	—	—
42	—	—	—

**E. coli* at 10^3 as test organism.
+Antibacterial activity present.
—Antibacterial activity absent.
±Slight activity.

In brief, the antibacterial action of whole human blood varies slightly from species to species and individual to individual in the organisms susceptible to its action, the dilution at which it is effective, the numbers of organisms which it will inhibit or destroy and the length of time it is active during storage under varying temperatures.

Studies on neutralization of the antibacterial action were made. As noted above, previous studies had shown gastric mucin to be effective in neutralizing the antibacterial activity of blood and plasma. A brief survey of other substances was made for comparison purposes. Glucose, gelatin and peptone were prepared in the concentrations shown (Table 9) and added in 0.5 ml. quantities to 0.5 ml. of whole blood. Varidase (Lederle) a mixture of streptokinase (fibrinolysin) and streptodornase (desoxyribonuclease) in a final concentration of 50 thousand units and 12.5 thousand units was added to blood. Wydase (Wyeth),

TABLE 9

*Neutralization by Mucin and Other Substances of the Antibacterial Action of Normal Blood**

Material tested blood tested	Sampling Time 1 hour	3 hours	6 hours	12 hours	24 hours
50 percent glucose					
rabbit	—	—	—	—	—
human	—	—	—	—	—
20 percent gelatin					
rabbit	—	—	—	—	—
human	—	—	—	—	4+
20 percent peptone					
rabbit	—	—	—	—	—
human	—	—	—	—	4+
Varidase†					
rabbit	—	—	—	—	—
human	—	—	—	—	—
Wydase‡					
rabbit	—	—	—	—	—
human	—	—	—	—	—
0.85 percent saline					
rabbit	—	—	—	—	—
human	—	—	—	—	—
5 percent gastric mucin					
rabbit	—	—	3+	4+	4+
human	—	—	4+	4+	4+

**E. coli* at 10^8 and 37° C.
†Streptokinase—Streptodornase (50 thousand units—12.5 thousand units per ml.) (Lederle.)
‡Hyaluronidase (75 TR units/ml.) (Wyeth.)
—No growth.
+Amount of growth.

a preparation of the spreading factor hyaluronidase, was added in a final concentration of 75 TR units per ml. to whole blood. As controls, physiological saline (0.85 per cent) and an equal volume of 5 per cent gastric mucin were added to separate blood samples. The test organism, *E. coli*, was added in a 0.1 ml. amount to each ml. of blood so as to give a final number of organisms at 10^3 per ml. Samples were removed for plating as described above during a 24-hour incubation at 37° C. Of the substances tested on both human and rabbit bloods, gastric mucin showed rapid neutralization by allowing growth of *E. coli* within 6 hours. With glucose and physiological saline, no neutralization was noted. Some activity was found with both gelatin and peptone in human blood. Growth was obtained with *E. coli* only at 24 hours with these two materials in human but not rabbit blood.

Further investigation on gastric mucin neutralization of the blood antibacterial action was made. Two commercial types of gastric mucin (Armour and Wilson) were available for test (Table 10). Four lots of Wilson and one of Armour mucin were studied. The mucin was prepared in a 5 per cent concentration by adding 5 grams per 100 ml. of water in a 500 ml. Erlenmeyer flask and allowing it to sit for 4–6 hours at 4° C. with intermittent shaking and agitation. The mucin was then sterilized by autoclave at 115° C. for 10–15 minutes. After setting the pH to 7.0 with sterile N/1 NaOH, the mucin was added asceptically to

TABLE 10

*The Neutralization of Antibacterial Action of Human Blood by Different Mucins**

Mucin	Sampling Time 1 hour	3 hours	6 hours	12 hours	24 hours	48 hours
80836 Wilson	—	—	3+	4+	4+	4+
83187 Wilson	—	—	4+	4+	4+	4+
82026 Wilson	—	—	4+	4+	4+	4+
82625 Wilson	—	—	4+	4+	4+	4+
Armour	—	—	3+	4+	4+	4+
None (control)	—	—	—	—	—	—

**E. coli* test organism at 10^3 level at 37° C.
—No growth.
+Amount of growth.

the blood or media for testing. The mucin was always tested for sterility before use. The addition of 0.5 ml. of 5 per cent sterile gastric mucin of each type to 0.5 ml. of defibrinated blood neutralized the antibacterial action of rabbit and human bloods for 10^3 *E. coli* cells incubated at 37° C. over a 48-hour period. The *E. coli* grew out in all instances within six hours.

Under similar conditions of test, a wide spectrum of organisms which were unable to grow in the presence of normal human blood (Table 3) were able to grow in the blood when gastric mucin was added. These results are summarized in table 3.

The level of mucin ability to neutralize the antibacterial action of whole blood for *E. coli* was determined. Table 11 shows

TABLE 11

*Effect of Dilution of Mucin on Inhibition of Antibacterial Action of Human Blood**

Mucin dilution	Sampling time 1 hour	3 hours	6 hours	12 hours	24 hours
1:2	—	—	1+	4+	4+
1:4	—	—	—	4+	4+
1:8	—	—	—	3+	4+
1:16	—	—	—	3+	3+
1:32	—	—	—	1+	3+
1:64	—	—	—	—	3+
0 control	—	—	—	—	—

**E. coli* at 10^3 level at 37° C.
—No growth.
+Amount of growth.

the data from a typical experiment. The 5 per cent mucin was diluted by two-fold steps through whole blood and 10^3 *E. coli* cells added per ml. When diluted as far as 1:32–1:64 in whole blood, the 5 per cent preparation of mucin still showed slight evidence of neutralizing capacity. Other studies with different samples of human blood suggested mucin activity as far out as 1:64 dilution of the 5 per cent mucin specimen or further on occasion depending upon the level of antibacterial action of the blood under test.

In experiments simulating blood culture of human blood, the effect of blood volume on the growth of organisms was determined. *E. coli* was again the test organism at the 10^3 level of organisms. The blood was added in varying amounts to 30 ml. of nutrient broth and broth plus 5 ml. of 5 per cent gastric mucin

in blood culture bottles. Incubation was at 37° C. for 14 days. The resulting data shown in table 12 reveals that with large volumes

TABLE 12

The Effect of the Amount of Human Blood on Obtaining Positive Blood Culture With and Without Mucin*

Amount of blood added (ml.)	Broth	Broth and mucin
1	+ 1 day	+ 1 day
2	+ 1 day	+ 1 day
4	+ 2 day	+ 1 day
8	+ 2 day	+ 1 day
16	— no growth	+ 2 day
24	— no growth	+ 2 day

**E. coli* as test organism added at 10^3 per sample of blood. 5 ml. mucin added to 30 ml. broth.
—No growth in 14 days.
+Growth in days.

of blood the organisms did not grow out as rapidly. In fact, the *E. coli* failed to grow within 14 days when 16 and 24 ml. of blood were added to the 30 ml. of broth. The *E. coli* grew out, however, in the presence of the gastric mucin. These findings suggest that large volumes of blood added to blood culture broth may delay or completely inhibit the growth of the infecting organism.

Summary

Fresh rabbit, sheep, and human bloods show antibacterial activity against a wide spectrum of bacteria.

The antibacterial action varies from species to species and from individual to individual in amount as to the dilution at which it is active and the types and numbers of organisms it will inhibit or destroy.

The antibacterial action is present in blood prepared with various anticoagulant procedures and in defibrinated blood.

Loss of activity on storage depends upon temperature and time of storage. In refrigerated blood, activity is lost slowly over a four-week period while at 37° C. is lost in a few days.

The range of activity of human blood as to dilution and numbers of organisms inhibited suggests a possible role of this inhibitory action in the delay of obtaining positive cultures and in the number of negative blood cultures from patients of suspected bacteremia or septicemia.

The antibacterial action of whole blood against a wide spectrum of organisms may be neutralized by gastric mucin.

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STUDIES ON GASTRIC MUCIN

IV. Gastric Mucin as an Adjuvant in Blood Cultures*

Frank B. Engley, Jr., Carl F. Page, and
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Numerous theories have been presented by Olitzki (1948) for the mode of action of gastric mucin as a virulence enhancing or resistance lowering substance. One of these suggests that gastric mucin neutralizes or inhibits the antibacterial action of normal blood and body fluids. Evidence has been reported by the author (1950) in support of this theory as one of the more important modes of action of mucin in experimental *Shigella dysenteriae* infections in mice. Other evidence (Engley, 1954) with numerous organisms reveals that normal bloods of humans as well as animals have a wide range of antibacterial activity in low dilutions. Some of the organisms susceptible to the blood action are those found in human bacteremias and septicemias. The antibacterial activity of the blood could be inhibited or neutralized by the addition of sterile gastric mucin. This evidence suggested a possible application for gastric mucin in routine clinical blood culture procedure.

Preliminary to the investigation of gastric mucin as an adjuvant in blood culture, a brief survey was carried out to find or choose a standard blood culture procedure. A brief survey (see references) revealed that a standard method for blood culture was not in use throughout the field of clinical bacteriology (Table 1). Variations were observed in the type of medium although each used an enriched broth such as brain heart infusion (Difco)[1] or trypticase soy broth (BBL)[2]. The amount of medium varied from 20–100 ml., while the container varied from 100–250 ml. flasks to vaccine bottles or screw-cap bottles of different sizes. The amount of blood inoculated also had a wide range, from 2–30 ml. Some sources suggested increased CO_2 tension for

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[1] Difco Laboratories, Detroit, Michigan.

[2] Baltimore Biological Laboratories, Baltimore, Maryland.

TABLE 1

Methods for Blood Culture

Reference	Medium	Amount of medium (ml.)	Container	Amount of blood (ml.)	CO_2 added	Temp. (° C.)	Incubation time (days)
Zinsser and Bayne-Jones, 8th Ed., 1939	Hormone broth or agar or Dougles broth or agar or glucose broth	100	250 ml. flask	10–20	No	37	..
John Sealy Path. Labs	Br. heart inf.	60–80	Sq. bot. (4 oz.) screw cap top and diaph.	5–10	No	37	7
Pedi. Bact. Lab., John Sealy Hosp.	Br. heart inf.	30	Sq. bot. (2 oz.) screw cap top and diaph.	10	No	37	7
Jordan and Burrows, 15th Ed., 1949	Br. heart inf.	50	100 ml. flask	10–20	No	37	7+
Todd and Sanford, 11th Ed., 1950	Nutrient broth plus ascitic fluid Br. heart inf.	100	250 ml. flask	5–20	No	37	10–14
Schaub and Foley 3rd Ed., 1949	Br. heart inf. and thioglycolate	100	250 ml. flask	5 to broth, 1 to thiogl.	No	37	6–7
Kolmer, 5th Ed., 1951	Tryptose phosphate broth	30	60 ml. army vaccine bot.	20–25	No	37	15
Conner and Mallery, 1951	Trypticase soy broth	50	B-D Vacutainer cult. bot. 100 ml.	20	Yes	37	14
Frobisher, 1949	2 percent bile broth	100	Flask	5	...	37	..
Burdon, 3rd Ed., 1947	Beef inf. broth	100	.....	5–10	Yes	37	..

TABLE 1—(Continued)

Methods for Blood Culture

Reference	Medium	Amount of medium (ml.)	Container	Amount of blood (ml.)	CO_2 added	Temp. (° C.)	Incubation time (days)
BBL, 1950	Trypticase-soy broth preferred and thioglycolate. Br. heart inf. gives some false negs. by limited growth promotion	40–50	.....	5–30	Yes	37	..
Difco Manual, 1948	Br. heart inf.	50	100 ml. flask	10–15	..	37	21
Smith and Conant, (Zinnser) 10 Ed., 1952	Beef inf. glucose fr.	50	Flask	15	...	37	10
Dept. Army Tech. Man. 8-227, 1951	Dextrose infusion broth	100	Flask	2–3/100 medium	...	37	..
Topley and Wilson, 3rd Ed., 1946	Na taurocholate broth	50–100	250 ml. flask	10	...	37	5
Lab. Meth. of U.S. Army, Simmons and Gentzkow, 1944	.....	100	250 ml. flask	2–3	...	..	..
Civil Defense Blood and blood derivative program, 1952	Thioglycolate broth	50	.....	5+	...	32–35	7
Lab. Meth. U.S. Navy, Bethesda	A basic broth	50	Flask or bot.	8	Yes	37	10
Microbiol. and Public Health; Sharp	Broth with sugar and brain	20	.....	10	...	..	..

the more fastidious *Brucellae* or other similar organisms. There was a reasonable unanimity of agreement on the temperature of incubation (37° C.) although one suggested 32–35° C. The incubation time also was inconsistent, with times ranging from 7–21 days. From these procedures we chose the type and amount of media, container, amount of blood, temperature and time of incubation which appeared most advantageous for conditions of test to obtain positive blood cultures.

Materials and Methods

A group of blood cultures from routine clinical cases of suspected bacteremia or septicemia was carried out in duplicate. In one series, 2 ml. of the suspected blood sample was inoculated into a two ounce bottle covered with a screw-cap rubber diaphragm closure and containing 30 ml. of trypticase soy broth (BBL). The other series was identical except for the previous addition of 5 ml. of sterile 5 per cent gastric mucin. The mucin was autoclaved at 121° C. for 10 minutes and the pH set with sterile NaOH to pH 7.0 after autoclaving. Sterility controls were carried out on the mucin after preparation and on representative mucin-broth bottles to insure against contamination.

The bloods were collected at the bedside by the technician and added directly to the bottles. In addition, the technician drew enough blood for her routine blood analyses and culture. Incubation of the cultures was for 14 days at which time they were subcultured for the last time and discarded. Subcultures were made routinely to blood agar plates for isolation of the organisms at 1, 3, 7 and 14 days. On occasion where the appearance of the blood culture suggested growth, the subcultures were made more frequently. The blood plates were incubated at 37° C. for 24–48 hours. All cultures were identified by colony characteristics, gram stain morphology, biochemical reactions and serologically, where necessary.

Results

A series of 191 routine clinical blood cultures from suspected cases of bacteremia or septicemia were run in duplicate according to the procedure outlined above. Of the 191 cultures, a total of 62 cultures were found to be bacteriologically positive using the results from the duplicate test bottles (Fig. I). The mucin broth yielded a total of 52 positive cultures while the plain broth yielded only 32 positive in the same group. The mucin broth yielded 20 more positive cultures than plain broth. However, there were 10 cultures positive in plain broth which were negative in mucin broth (Fig. II).

The findings of another laboratory with the same cultures (Fig. I) utilizing 5–10 ml. of blood in 50 ml. of brain heart in-

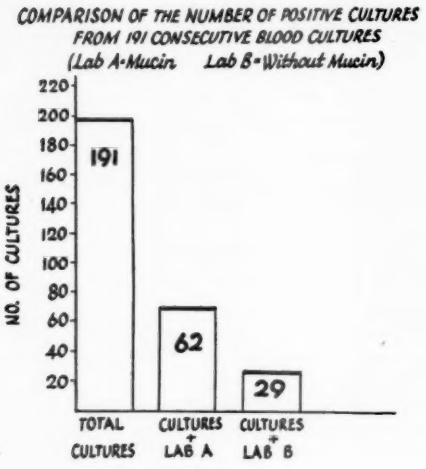

FIGURE 1

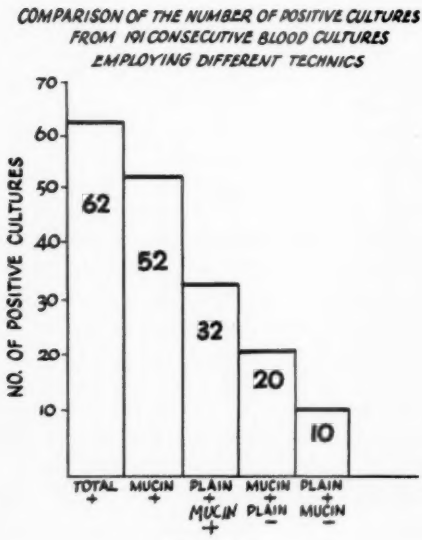

FIGURE 2

fusion resulted in 29 positive cultures in comparison to 62 cultures obtained with the broth and mucin broth technics. As noted above from figure II, a total of 42 of the 62 cultures were obtained with broth and 52 with mucin broth.

The organisms isolated in the series of blood cultures are listed in table 2. A large number of *Staphylococcus* and gram

TABLE 2

Organisms Recovered From Blood Culture by Mucin Technic

Staphylococcus albus	15
Staphylococcus aureus	7
Aerobacter aerogenes	11
Escherichia coli	2
Paracolobactrum aerogenoides	2
Paracolobactrum coliforme	1
Pseudomonas aeruginosa	2
Alcaligenes fecalis	12
Diplococcus pneumoniae	3
Streptococcus viridans	1
Corynebacterium spp.	2
Neisseria spp.	4

negative rods including *Alcaligenes*, *Aerobacter*, *Paracolobactrum* and *Pseudomonas* were isolated. There appeared to be no real difference in the types of organisms isolated in mucin broth or in plain broth. Some of these might be considered as possible skin or other contaminants. However, repeat cultures for the most part substantiated the presence of these organisms in the blood. Some repeat cultures were obtained only after antibiotics or chemotherapy had been instituted. In one or two instances, the organism isolated from blood culture, *Aerobacter aerogenes* for example, was isolated at autopsy. The data suggest that the addition of gastric mucin may make the blood culture technic a very sensitive test. The mucin-broth may be isolating organisms from the blood stream that are present as chance transient bacteremias. However, the mucin may be neutralizing the antibacterial activity of the blood to allow the causative agents of bacteremias and septicemias to grow out from strongly inhibitory bloods. This neutralizing effect of mucin may be important especially when large quantities of blood (10–20 ml.) are added to small amounts of broth (30–60 ml.). The resulting dilution of the blood by the broth is still within the antibacterial range of many blood samples. This antibacterial activity of blood may

delay or completely inhibit the growth of organisms from the blood.

A striking difference was observed in the time when positive cultures were obtained in cultures with and without mucin. The data are presented graphically in figure III. A greater percent-

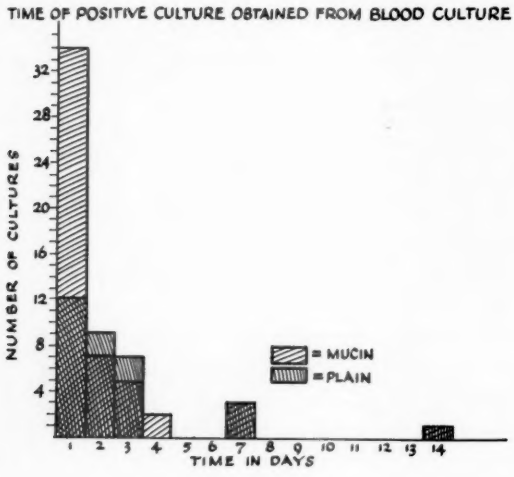

FIGURE 3

age of positive cultures were obtained with mucin in the first one or two days of incubation than without mucin. It appears that mucin allows the organisms to grow out more rapidly. After the greater percentage at first in mucin the plain broth cultures seemed to give equal results through the 14 days incubation period.

In addition to increasing the number of positive cultures and decreasing the incubation period it was also observed that the subcultures from the mucin bottles gave more luxuriant growth. All plates were streaked with a standard loop. The resulting growth on blood plates were graded as to amount of growth by a plus system. Luxuriant growth (many colonies, large size) 4+, good growth 3+, fair growth 2+, and poor growth (very few colonies, small size) 1+. Figure IV compares the growth obtained on the subculture blood plates. It will be noted that

the great majority of subcultures from plain broth gave almost equal numbers of the four different grades of growth. A comparison of the amount of growth was most striking, for the mucin subcultures gave much more luxuriant growth and larger number of colonies than growth on subculture from plain broth.

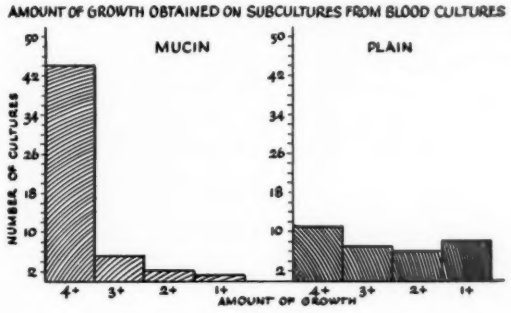

FIGURE 4

The increase in number of positive cultures obtained in plain trypticase broth over the findings of a second laboratory could be due in part to the medium or the amount of blood added to the broth. Experiments with simulated blood cultures (Engley, 1954) have shown that normal blood antibacterial activity may be present in dilutions up to 1/10 or greater. It is suggested that where volumes up to 10 ml. are added to broth volumes of 50 ml. that the blood may exert a strong inhibitory action on organisms present. In these studies, 2 ml. of blood were added to 30 ml. of broth thus diluting the antibacterial activity to a great extent. The second laboratory added 5–10 ml. of blood to 50–60 ml. of media.

Summary

A procedure has been described utilizing gastric mucin as an adjuvant in routine clinical blood culture.

In a short series of cultures (191) mucin broth provided a larger number of positive cultures (52 as compared to 42 with plain broth).

Comparison of results with another laboratory suggests the importance of diluting out the antibacterial action of blood in large volumes of broth.

The cultures in mucin broth grew out more rapidly than the plain broth, especially within the first several days was this observed.

The growth of subcultures from the mucin broth was more luxuriant than from the plain broth.

The results suggest that gastric mucin may provide an adjuvant effect in blood cultures providing more positive cultures by neutralizing the antibacterial activity of the blood and allowing the organisms to grow out more rapidly and more luxuriantly.

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MYTHS IN PSYCHOTHERAPY

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All societies have their anxieties and have attempted to work out—not always through deliberate planning—ways to alleviate these anxieties. And all societies, not only primitive ones, have their medicine men, their healers and shamans, their soothsayers, and magicians. Around the medicine-man there develops an aura of mystery, perpetuated in part by the wishful thinking and strivings for succor of those who come to him for help, and perpetuated also in no small measure by the medicine-man himself for reasons of self-aggrandizement as well as to fulfill his own grandiose and omnipotent strivings. The magic of the medicine-man is spun through rituals and ceremonials; and perhaps the more outlandish the ritual to be performed and conformed to, the greater the will to believe and to be healed.

Such rituals may take the form of incantations and litanies, of strange syllables intoned in measured and inaudible cadences, and the ceremonials will emphasize the disparate levels of the medicine-man and those who seek his healing. In time the medicine-man, whatever his original skepticism about his own powers, may succumb to the hazards of his occupation and the projections of his beseechers and formulate principles to buttress his ritualistic procedures. If the practice of witchcraft prospers, schisms may develop among the medicine-men and sub-groups begin to form, each convinced of the divinity of its system of beliefs and rituals. Procedures for the selection, training and supervision of new medicine-men are adopted, oaths of fealty are extracted from the new practitioner, and covenants are entered into binding the members of the medicine-man's cult. If all medicine-men are attacked, there may be a period of solidarity coinciding with the period of attack; if, however, practice flourishes, splinter groups may continue to form. Each new group may adopt some new principles or reaffirm allegiance to a medicine-man of old; the school of reaffirmers may reassure

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themselves by daily prayers to the progenitor's cave-wall markings and by repeating his movements exactly as he performed them. And so mythology about legendary figures and legendary rituals develops. If the progenitor had developed an arthritic limp from sleeping on the damp ground, his followers would limp also in the belief that limp and successful healing were somehow causally correlated.

The psychotherapist is the medicine-man of our time.

Myths in Psychotherapy. A myth, say the sociologists, is a collective belief that is built up in response to the wishes of a group instead of to an analysis of what the belief pertains to. A myth is also a legendary story concerning some superhuman being or some alleged person or event, or a deity or demigod.

In an area such as psychotherapy, which touches human psyches at least as strongly as these are affected in religion, and in political-social reform movements, it is not surprising to find a wide variety of mythologies and fantasies about the practice of psychotherapy. Just as primitive peoples rely heavily upon mythology and incorporate many of their myths into rituals and ceremonials, so do we living in a 20th century industrial society have our myths and rituals. Even psychotherapists, no matter how many hours they have spent on the couch or in the armchair, have their share of myths about themselves as individuals and as therapists, about their patients, about theories of psychotherapy, the psychotherapeutic process, and the nature of change or cure in psychotherapy.

The genesis of such myth-development is suggested by Whitaker and Malone (1953). The therapist tends to accept the projections of his society, of his colleagues, and of his patients. He idealizes his role in terms of these projections and begins to behave omnipotently.

It is this feeling of omnipotence which leads to the development of a host of other myths.

This brief paper, which does not pretend to be an exhaustive or scholarly exposition, but only a provocative one, will discuss some of these myths. The many obvious and blatant misconceptions which the public holds about psychotherapy do not belong here, for it is not our purpose here to make a case for psychotherapy. We are instead concerned with those myths of psycho-

therapy which obfuscate therapeutic progress and limit the growth of the therapist and patient alike.

The Psychotherapist's Most Dangerous Myth. Perhaps the most dangerous myth of all which the therapist carries with him into the consulting room is the legendary notion about the well-analyzed person—therapist or patient—and the so-called completed analysis. A therapist is expected to be so well analyzed as to be "cured." It is hard for a therapist to admit to himself, to his patients, and to his colleagues that he has so-called residual problems. He is especially likely to conceal his feelings of anxiety and to be extremely cautious about confessing to any physical illness. Physical illness has to be blatantly respectable in order to avoid any possible opprobrium of "psychosomatic." Gastro-intestinal illness, cardio-vascular diseases, and allergies are strictly taboo; even the common cold is suspect. The psychotherapist who develops any of these illnesses fears that colleagues and patients who learn about this will wonder about him and doubt his psychic strength. Even worse is the tendency for the psychotherapist himself to regard such physiologic reactions to stress as irrefutable evidence that he is not altogether qualified to help his patients.

This conception of the therapist—analyzed to the point of omnipotence—is frequently harbored as a myth of the patient, who often has a need to see the therapist as all-powerful and all-knowing, as a "socially competent" person, as one who is never sick, who is never shy or troubled or anxious, and who is always able to handle his problems and the problems of other people completely and competently.

These are powerful projections for the therapist to contend with, especially if he also entertains some skepticism about his maturity and his own analysis—perhaps something he needs to *suppress* (for who can believe that a well-balanced therapist *represses* anything?). The therapist is now ready in turn to project such power onto other therapists. He comes to believe that they produce miraculous character changes in their patients, that these patients are completely and permanently sanforized, and that he should and must achieve similar spectacular results. And so he tends to set up unrealistic therapeutic goals, perhaps unconscious goals, for his patients and therefore for himself.

It is very difficult to determine what is really expected in the refurbished personality of the well-analyzed patient. Obviously everybody agrees about improved functioning in work, in love, in social life, and there is also some agreement as to creativity and productivity. But underneath all this is a legend, or rather a legendary figure of W.A.M., "the well-analyzed man."

What does this ghost seem to look like? So far as I am able to make out, he is supposed to be sort of flat. He doesn't get too euphoric or too depressed. He is not impulsive. But he is flexible enough to indulge in a smile occasionally after he has evaluated a joke and analyzed whether his response to it is neurotic or not. He is not supposed to have any body illness, for this must at once bear the opprobrium of "psychosomatic" (a much misunderstood and misused term). Thus, he cannot even enjoy the common cold, since *it* is probably psychosomatic; so instead of staying in bed for a day or so indulging a normal lower-class feeling of dependency, he drags himself around convinced that some conflict has set off the sniffles. The same is true of any accidents. (In all of this mythology please note how easy it is to lose sight of the point that as human organisms we are capable of reacting to stress in only a limited number of ways, that we react as total organisms, and that the limited number of adaptations or responses we can make is infinitely smaller than the tremendous number and complexity of stimuli to which we can be exposed.)

About the only respectable physical impairment the well-analyzed man can have is a fracture on being hit by a bus, provided the driver was drunk and ran his bus onto the pavement and into the backyard where the W.A.M. was sitting under a tree reading Freud's *Collected Papers*.

Apparently we forget that psychoanalysis—even the best brand done by the best therapist—is after all a kind of repair job, although it may be a good one; that perhaps the healthiest people are those who, like Topsy, just "growed" that way. As a colleague said at a meeting at the Post-graduate Center for Psycotherapy where problems of evaluation of therapeutics results were being discussed: "The bones are just the same, although maybe the flesh looks a little different."

This looking a little different should not be belittled. Therapeutic changes can be and are tremendously significant for the

individual who experiences the changes. And it is his subjective feeling which counts, even though his friends and colleagues may not see too many changes in him. Perhaps therapists have too readily accepted the projections of analysands and their friends that the analysand shall in truth have become reborn.

The Myth of Omniscience. Closely allied to the feelings of omnipotence is the myth of omniscience. This, too, is projected onto psychotherapists by patients, whose need is strong to believe that the therapist knows every moment what they must be thinking and what is happening in therapy. In addition, whether through some kind of perceptual distortion or through a process of osmosis, the therapist too may get the idea that his teachers, his supervisors, prolific analytic writers, and people who give papers at scientific meetings really know every minute of the time what is going on in the therapeutic situation. It is certainly true that the more competent the therapist, the more he is aware of what is going on in the patient, in himself, and in the two-group therapy situation. But this awareness is likely to constitute only a small fraction of the verbal and non-verbal transactions that go on in a single therapeutic session.

To illustrate this need for omniscience, take dream interpretation. There is no question that some therapists are more skilled and gifted than others in handling dream material. But it is doubtful that even the best psychotherapist can always fully and completely analyze every dream. As Freud pointed out in describing the "Employment of Dream-Interpretation in Psycho-Analysis" (1912): ". . . the analyst should recognize that in cases of severe neurosis any elaborate dream-productions are to be regarded as, theoretically and in the nature of the case, incapable of complete solution . . ." (p. 307). And further: "It often happens . . . that the best way to complete the interpretation of a dream is to dismiss it and to devote attention to a new dream, which may contain the same material in perhaps more accessible form" (p. 308).

There are also other aspects of the therapeutic situation where omniscience can operate. How often do patients, whether in accusation or adulation, say: "I guess you know *what* I am thinking better than I do." One of the best antidotes I ever received for this kind of omniscient thinking occurred in my own analysis. It was during a difficult period in the analysis when I

was confused about what was happening. I accused my analyst of holding out on me, of knowing something he wasn't communicating to me. To my amazement—and certainly, later, to my great benefit—he answered somewhat in this vein: "Here I am beating my brains trying to figure out what is going on and you are accusing me of withholding knowledge I do not at this time have." To me this was at first a stunning blow. To think that not only did *I* not know what was going on at this difficult period, but that the analyst also didn't have the answer! It was only later that I came to appreciate his honesty and my own needs for projecting omniscience.

Patients can thus use their dependency longings. If the therapist is all-knowing and also omnipotent, he can take care of the patient, and the patient doesn't have to take any responsibility for his life.

Insecurity about his analysis, about his training, about his status among his colleagues, about his case load, about referrals—all these may add to the therapist's anxiety. Judd Marmor (1953) has said that the feeling of superiority of the therapist may be a defense against anxiety arising out of his feelings of inadequacy. The seductive adulation of one's patients is difficult to resist.

Transference. There are also myths about the phenomenon of transference. When used as a myth, transference can be the therapist's best dodge in dealing with a critical or complaining patient. As David Rioch (1951) points out: "The theory that all the performances of a patient with a therapist are in terms of patterns of his earlier life has been of great value in relieving the anxieties of the therapist and of the patient, and with the relief of anxiety, permitting a more mature and comprehensive consideration of the problem. This theory, however, has been grossly overworked. I have not known of any instance of a psychotic or neurotic patient responding in a situation to something which was not there, though frequently the observer has to scrutinize not only the immediate situation but its time-course and his own participation, to determine that aspect to which the patient is responding."

According to Rioch, even hallucinations are symptoms—that is, defenses against anxiety—and the hallucinations themselves rather than their bizarre form or content constitute a response

to real factors in the situation. Rioch feels that it is thus important for the therapist to look into his own behavior and to discuss his own attitudes with the patient to clarify the real factors the patient is dealing with in his interaction with the therapist; and if the therapist is not comfortable enough to do this, it's time for him to consult a colleague.

Counter-Transference. And if there are myths about transference, why shouldn't there be myths about counter-transference? In psychoanalytic literature, much stress is placed upon certain of the therapist's transferences on the patient. These are called counter-transference simply because they go the other way (Mullan, 1953). Nevertheless they are transferences or distortions just the same. One sometimes wonders whether this is another way of preserving the therapist's omnipotence: a given phenomenon in a patient is called transference; the same phenomenon in the therapist is given the more exhalted name of counter-transference. It's like calling a shovel a hand-spade when you use it and a shovel when a day-laborer uses it.

At any rate, in the literature reference is made to certain feelings of the therapist toward the patient which are carry-overs from the therapist's past relationships. For the most part the literature is concerned with such transferred feelings as sexual attraction, competition, anger, etc. Here again David Rioch has made a keen observation. He doubts that these feelings occur as often as claimed in terms of former relationships in the life of the therapist. Instead, he sees many of these occurrences as temporary and likely to reflect some anxiety arising from the therapeutic situation, especially when there is some unclear problem. He sees counter-transference as the effect rather than the cause of the difficulties. If this be so, it then becomes necessary for the therapist to examine what is going on in *his* life at the moment as well as the sequence of events in this particular therapeutic situation. Rioch believes that one of the important clues in discovering the therapist's anxiety lies in the therapist's relation with his colleagues. Says Rioch: "When the therapist's security in his group status is threatened, there are very frequent repercussions in the patterns of relationships with his patients. This is in part related to the fact that one of the commonest forms of counter-transference is that in which the therapist is playing

the role of 'what the therapist ought to be' and is fitting the patient with the proper theory . . ." (Rioch, 1951).

The Myth of Theory and Interpretations. The sub-title of this section ought to be: "A Theory in Search of a Patient." During the past few years we have been exposed to a spate of articles, papers and books on theory. This is highly commendable: there ought to be theories about human behavior. But there is a dearth of theory about the therapeutic process itself. (One of the notable exceptions is the stimulating work of Whitaker and Malone (1953), *The Roots of Psychotherapy.*) The myth seems to be that if you have the right theory—or rather, of course you *have* the right theory—then all you have to do is to time your interpretations in line with your theory and the patient will eventually gain insight, "work through," and attain genital primacy, maturity, or whatever criterion of "cure" you use. And case materials are advanced to support this myth: such-and-such were the dynamics, the genetics, and such-and-such interpretations were made: Q.E.D., patient recovers.

If this line of reasoning is valid, if a theoretical position, leading to interpretations in line with the theory, produces these recoveries, then all we have to do is to study the results of therapists of different schools, equating the therapists of course in terms of experience. This ought to show us which theories are valid. But whatever systematic research has been done on the subject, especially the work of Fred Fiedler (1950), seems to suggest that, by and large, experienced therapists of whatever school produce good results and that inexperienced therapists produce results that are not as good; also that what the good therapist does in the therapeutic situation and how the patient experiences the therapist and his therapeutic techniques is markedly similar from school to school in the case of good results.

Sigmund Freud (1904) said: "There are many ways and means of practicing psychotherapy. All that lead to recovery are good. . . ."

Now either this means that the competing theories and their resultant interpretations are equally good or valid, or that good therapists of whatever school can do good work. As psychologists who have all had at least some training in research and scientific method, we would be reluctant to accept this equivalence of competing theories or hypotheses. As researchers, we should

have to entertain the hypothesis that patients can get well—or not—despite the theory. In short, it is the therapeutic two-person group situation which is the vehicle of treatment, and it is the therapist who is the instrument of treatment—and not the theory. What we need is a theory of *psychotherapy* rather than more theories of psychodynamics. We have to study the therapist as a "curative" agent rather than his words. Dr. Lewis Wolberg has estimated that probably one out of every three responses of a good therapist is in some way a technical error; perhaps this impedes therapeutic progress and perhaps it doesn't. Patients would seem to get well because, maybe for the first time in their lives, they have established a certain kind of relationship with another individual.

Sometimes we are fooled into believing the myth of theory when patients report dreams and fantasies in which we find corroborating evidence for our interpretations. If this evidence is examined further, we shall find that we have really suggested to the patient *our* theories of his unconscious; or, to put it differently, the theory of the therapist becomes the patient's unconscious. A therapist who employs the construct of the fecal penis is more likely to find his patients dreaming so often of feces, and a Jungian patient may dream more often of fire, etc.

Interpretations are supposed to lead to insight. Jules Masserman (1953) described insight as "representing that mutually happy state in which the patient professes acceptance of the current formulations of his therapist." Masserman says this led him to "relinquish the phantom of verbal 'insight' as merely a narcissistic projection of the therapist's own intellectual brilliance, focused on the patient purportedly to illuminate his path but really casting shadows that as often confuse and mislead him. . . ."

The therapist's need for omniscience and omnipotence projected on him by his patients, by his colleagues (a sub-culture), and nurtured by his own residual conflicts may result in his tendency to operate along the lines of self-fulfilling prophecy. He finds what he sets out to prove. The saving grace is that, despite such theoretical mythology, many competent therapists nevertheless help their patients by their warmth, their interest, their non-judgmental and non-moral permissiveness.

The Myth of Psychosomatics. Another myth—which is sometimes held more strongly by non-medical therapists who have had experimental backgrounds, although it is also held by medical therapists as well—is the psychosomatic myth. The confusion stems partly, as Schick (1953) has pointed out, from mistaking psychodynamics for etiology; that is to say, psychological factors may have something to do with the motivations associated with an organic symptom or illness, but it is obvious that not every human organism responds to stress in the same way. Why one person gets a coronary occlusion and another an ulcer is not quite so easily determined as the profile-makers would have us believe as they go about setting up an ulcer type, an arthritis type, an asthma type, an accident-prone type, etc.

The fallacy of many psychotherapists consists in ruling out constitutional and hereditary factors in their effort to combat old-fashioned devotion to instinct. This is surely no place to debate the nature-nurture controversy, but it ought to be said that man's destiny is not only a matter of his childhood and later-life experiences but also of his parents' genes as well. Thus Schick (1953) for instance, doubts the psychotherapy can *cure* such an illness as essential hypertension, since he views this as a constitutional disease entity; he admits, however, that psychotherapy can provide some symptomatic relief.

I recall a male hypertensive patient whose mother and father had died of coronaries. He had three brothers living who had had coronary occlusions; a fourth brother had hypertension and had been on a salt-free diet for years. The patient entered therapy with anxiety states, and with hypertension; included among his fears was the fear of a coronary. (I might add that he was a physician who had specialized in cardio-vascular diseases.) He seemed to utilize psychotherapy as a positive growth experience, and it was terminated by mutual agreement when he reached the point where he seemed able to continue to grow and to cope with his problems relatively free of symptoms. He even passed an important health examination. He had made some important work adjustments and had somewhat improved his family situation, although he recognized that there were some as-yet-unresolved problems there.

Six months later he had a coronary. Obviously therapy did not prevent the heart attack he had feared. He was, however, able to

cope with the long hospitalization, the convalescence and the gradual resumption of almost-normal functioning.

To posit an "organ language" or any other psychosomatic concept based on psychodynamics *alone* is to neglect many essential factors (such as costitutional factors) in the etiology or casual sequence of any disease entity.

I do not mean to imply that many organic symptoms are not diminished or eliminated in the course of psychotherapy, for this is an everyday occurrence in practice. But the dragon that needs to be slain is the one which breathes the *same psychodynamic profile* into every case having a similar organic symptom. As Edward Stainbrook puts it: "Since pathogenic and stressing conditions are infinitely variable and human adaptive reactions are limited, it is obvious that similar adaptive reactions will be produced by quite different patterns and situations of stress."

It should also be clear that this is no attempt to negate the symbolic meaning in many symptoms. For one can easily translate these body-symptoms into commonly used expressions: "I can't stomach that" (vomiting); "I can't swallow that" (globus hystericus); "It sticks in my craw (dyspepsia); "To swallow one's resentment" (ulcer); "It gives me a pain in the neck" (torticollis).

Myths of Technique. Space will not permit extensive discussion of myths about technique. Some techniques in psychotherapy are used not because they inherently advance the therapeutic process but because they have become rituals and ceremonials which are perpetuated as vestigial remains without regard to their origin and present function. For example, no budding psychotherapist would think of furnishing a new office without including a couch; nor would any self-respecting *New Yorker* cartoonist, movie or stage director depict an analyst's office without one.

The myth seems to suggest that there is something "therapeutic" in the couch itself. A careful check of upholsterers will prove that the fabric of an analysist's couch is no different from that of anyone else's couch. To Gertrude Stein, a couch is a couch is a couch.

Since this article of furniture has no built-in therapeutic device, the question arises as to why the couch? The best answer may be found in Freud's (1913) own reasons: "Before I conclude

these remarks on beginning the analytic treatment a word must be said about a certain ceremonial observance regarding the position in which the treatment is carried out. I adhere firmly to the plan of requiring the patient to recline upon a sofa, while one sits behind him out of his sight. This arrangement has an historic meaning; it is the last vestige of the hyponotic method out of which psychoanalysis was evolved; but for many reasons it deserves to be retained. The first is a personal motive, one that others may share with me, however. I cannot bear to be gazed at for eight hours a day (or more)" (p. 354). Freud adds a few additional reasons connected with free association, transference and resistance, and he concludes by remarking: ". . . I know that many analysts work in a different way, though I do not know whether the main motive is the ambition to work in a different way or an advantage they gain thereby. . . ."

So Freud's use of the couch, which he describes as a ceremonial and a vestige of hypnosis, indicates that his primary motive was the personal one of not wishing to be gazed at. Certainly for many therapists the couch—as well as other classical techniques—has become a vestigial inheritance. One wonders whether its use as automatic *ceremonial*—the patient supine, the therapist sitting above and behind him out of sight—promotes the dependence of the patient, and the feeling of superiority of the therapist.

Therapists who use the couch exclusively, and therapists who use the couch and armchair alternately, claim a difference in the patient's productions. This is probably so—but it needs to be demonstrated what therapeutic function the difference serves.

It must be observed, however, that some therapists report that *their* responses are different when the patient is on the couch from what they are when patient and therapist are in a face-to-face position. One would have to know what *this* difference means and whether *it* promotes therapeutic growth.

One cannot quarrel with therapists who feel that they are under less tension when their patients recline, facing away from the therapist. Nor is there any quarrel with the therapist who uses the couch with a particular patient because the patient can talk more freely while on the couch. (Nor is there any quarrel with the therapist who uses the couch for himself, to snooze on when he has a cancelled appointment.)

These reasons are functional and straightforward. They do not, however, eliminate the possibility of increasing, because of the upper-lower positions of therapist and patient, the omnipotence and omniscience of the therapist as projected on him by the patient and as somewhat self induced because he does not have to "face-up" to the patient. It should not be implied that the face-to-face situation eliminates the hazard of omnipotence, for it doesn't do so of itself. But it is possible that the face-to-face situation makes it less easy to hide behind one's mask of authority. The myth I refer to inheres in the ceremonial use of the couch per se *as an indispensable therapeutic technique* and as a ritualistic vestige from the days of hypnotism.

Referring to technique in general, Masserman (1953) has wryly commented that "the therapist, too, glories in his own illusions that he can, solely, by the gaze of his eye or the somber spell of his voice, reverse time and space into 'regression,' and command illness begone. . . . It is an alert hypnotist who knows who is hypnotizing whom; here we may add that it is also a wise therapist with any technique that knows who is gaining most unconscious satisfaction from the treatment, especially when the latter becomes unnecessarily dramatic, lucrative, or prolonged."

The Myth of "Cure" and Goals in Psychotherapy. The myth of "cures" in psychotherapy is one of the most pernicious. It exists in part because of the dearth of data evaluating the effects of psychotherapy. But it is perpetuated also because of the human tendency for psychotherapists to talk about their successes and to dissociate their not-successes. It is unusual to hear a clinical paper which reports a therapeutic failure. Like fairy-tales, most clinical papers lead you to believe that the patient lives happily ever after. Hollywood and Broadway elaborate this only slightly: the patient and the therapist live *together* happily ever after.

The myth of "cure" leaves unanswered many questions which only solid research can answer. Can anyone estimate the proportion of patients in private practice who are cured, helped significantly, helped somewhat, helped not at all? This has been a carefully guarded trade secret. Our most likely beginning in the evaluation of psychotherapy will have to be made in clinical treatment centers.

In advance of such research, clinical experience dictates a much more modest claim for the therapeutic goals of psychother-

apy. Masserman (1953) is willing to define psychotherapy as "*any* procedure available to the ethical practitioner that helps the patient become happier, more creative, and better adjusted to his familial and social milieu."

As for "finished cures," Freud (1912), in advocating the analysis of potential psychotherapists, said: "Such analysis of a person who is for all practical purposes healthy will naturally remain uncompleted. Whoever knows how to appreciate the high value of the self-knowledge and increase in self-controls so acquired will afterwards continue the analytic examination of his own personality by a self-analysis, and willingly recognize that, in himself as in others, he must always expect to find something new . . ." (p. 329).

Later, in discussing goals for the patient, Freud (1912) wrote: "It is but a natural ambition for him [the therapist] to endeavor to make something specially excellent out of the person whose neuroses have cost so much labour, and to set up high aims for these impulses. But here again the physician should restrain himself and take the patient's capacities rather than his own wishes as his standard. . . . A physician must always be tolerant of a patient's weakness, and must be content to win back a part of the capacity for work and enjoyment even for a person of moderate worth. Ambitiousness in the educative direction is as undesirable as in the therapeutic" (p. 331).

Therapy should be viewed as only a phase in the process of growth of the patient and the therapist (Marmor, 1953). Therapy doesn't "cure" anyone, and it doesn't make children into adults (Whitaker and Malone, 1953). Therapy instead provides a profound emotional "unbinding" experience which allows the individual to continue to grow. Thus conceived, analysis is a never-ending life-long process for patient and therapist alike.

The Myth-Producing Eras in the Professional Life of the Therapist. My hunch is that if one holds personality factors constant, the most prolific myth-producing periods in a psychotherapist's professional life are these:

Period 1. During the last half of his didactic training and for a short time after its completion, the reliance on dogma is understandably strong. Psychotherapist-trainees are particularly prone to the mythology of omnipotence: they project it onto their supervisors and teachers and in turn have it projected onto them by

their patients. Some psychologists, moving, as they have done, from research and experimental psychology into psychoanalytic psychotherapy may take on the semblance of a religious conversion. Guilty about having given up the old religion (of scientific method, etc.), the new convert may tend to over-believe the new, more speculative religion. If he becomes anxious about his clinical performance—and which therapist-in-training has not experienced such anxiety?—the comfort of dogma may be great indeed.

Period 2. If he goes into practice upon completion of his training or somewhat before this, the therapist's mythology will depend on such factors as the success he has as measured by the number of referrals and the degree to which his patients arouse anxiety in him. During this period, the anxieties may be strong and mythological omnipotence and omniscience can easily be projected onto more experienced therapists. This second period, if it is overcome and the practice flourishes, is likely to be followed by the "success period."

Period 3. The "success period" may include strong ingroup ties with one's theoretical school, socialization with the members of one's sub-culture group—that is, other psychotherapists. Financial success may lead to the acquisition of material and nonmaterial sub-culture class symbols: these have to do with the kind of car one drives (convertibles preferred), how one spends one's vacation, the kinds of parties one gives, the clothes worn by the therapist and his wife, where one lives, and the way one spends his leisure time.

It is during this period that the therapist may become hopelessly enveloped in mythology about himself and his school. Working in isolation—for even though he "sees" people all day, psychotherapy is essentially a lonely occupation—and having become more skilled in handling patients, the therapist can succumb to their adulation. This, plus his competition with his colleagues, may lead him to believe, as Marmor (1953) pointed out, that his own particular brand of therapy is best. Omniscience and omnipotence reign supreme.

Now obviously this doesn't and need not happen to every psychotherapist, although the siren of omnipotence is seductive. This "success" period may, on the other hand, cause considerable conflict for some therapists, leading them to a reinvestigation of

how they have been thinking, operating therapeutically, and living. It's about this time that they may need to re-evaluate their own analyses, even to the point of surreptitiously dropping around to "consult" with their own analysts again.

Period 4. Too many psychotherapists, however, remain in the sublime state of omnipotence—God and the business cycle willing—for the rest of their professional lives. These are the greatest, and most dangerous, myth-perpetuators.

However, the best of the old practitioners in their 60's and some in their 70's are like some of the best old painters and sculptors who have passed through the earlier phases of devotion to technical craftsmanship and detailed representational phases of art. They have narrowed down to— no, they have broadened out to—only the essentials, like the line drawings of Matisse, Picasso's lithograph of a bull, and Roualt's later paintings. The mellowed practitioner has given up the reliance on tricks of technique, and most of the mythology. He even attends fewer meetings and prefers to have a car with a steel top or to take taxis. Maybe because he is no longer ambitiously striving or strongly libidinous, he can allow himself the luxury of being less omnipotent.

Other Myths. Other myths come to mind which I shall merely list, such as: If you "lose" a patient, you are a poor therapist; Unless you succeeded in looking through a transference block with the patient—that is, if he does not complete his analysis with you, and instead goes to someone else—it's a failure; If the therapy isn't going well, it's because the patient is in resistance; If the patient, after therapy, has a relapse, its because his analysis wasn't completed; A didactic analysis is different from a therapeutic analysis; The therapist ought to be completely anonymous, a mirror, a mystery; Where you have helped to bring about a good result, the patient will be ever grateful and will become your friend; and in your old age you will be visited every Sunday by droves of happy ex-patients.

Every therapist regards his own personal analysis as having been successful and has a good feeling about, if not a relationship with, his own analyst.

In concluding this recital of mythology, it would be a disservice not to repeat that much good psychotherapy is being done by good psychotherapists, and that good psychotherapists can probably

be distinguished from bad ones. At various times in their professional careers all psychotherapists succumb, if only in small measure and only temporarily, to one or another of the "myths" here discussed. But my hypothesis would be that better psychotherapy is done by those therapists who have been able to give up most or all of these myths as well as by those who are constantly coping with them.

Silverberg (1952) has advanced as a replacement for omnipotence the concept of *partipotence*, by which he means only partial mastery. Even the partipotent attitude must not be rigorously enforced or expected, for the healthiest can achieve it only through bitter struggle. Relapses, Silverberg points out, may occur in which one falls into the dichotomous all-or-none. He sums up his criteria for mental health as including these qualities: "competence in dealing with familiar situations; courage and resourcefulness in meeting new ones; the wisdom and humility to know one's powers and their limitations. Mental health seems to equate with qualities of maturity: reliable competence as distinguished from rashness and bravado; and humble wisdom as distinguished from arrogant ambition and intellectual conceit. Poets and philosophers throughout the ages have told us much the same thing" (Silverberg, 1952).

If only the psychotherapeutic myths of omnipotence, omniscience, and infallibility could be dispelled by humility and self-analysis!

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BACTERIOLOGICAL PROBLEMS IN MAINTAINING THE CHRONIC NEPHRECTOMIZED DOG*

FRANK A. GIGLIO, ROYAL B. LEA, AND CARL F. PAGE

The loss of the functioning kidney due to disease can be a rapid cause of death and such disease processes are not uncommon. This fact has led numerous investigators of renal physiology and all its ramifications to work with nephrectomized experimental animals, *i.e.*, the dog, cat, rat, and rabbit.

Maintenance of the nephrectomized dog in a good physiological state is a major problem. The situation duplicates that found in the patient who has lost kidney function due to such disease processes as fulminating acute glomerulonephritis (acute postinfectious hemorrhagic nephritis). Using various techniques, investigators have been able to keep nephrectomized dogs alive for varying lengths of time. Winternitz, *et al.*, in 1940, and Rodbard in 1945, reported survival time of two to seven days when no means of substitution for renal function was used. Grollman and his group reported in 1951, that survival time could be extended as much as six days by restricting salt in the diet. Grollman in 1949, and Leonards and Heisler in 1951, reported further increase in survival time up to twenty days by use of the artificial kidney. Bliss and his group had reported as early as 1932, a similar survival time by use of the peritoneal dialysis technique. This latter technique allowed Grollman in 1951, to maintain one dog for sixty-nine days. This dialysis technique began to gain favor and other workers quickly adopted it. In 1954, Houck and his co-workers reported having kept one dog alive for one hundred and eleven days before sacrificing the animal due to a chronic skin condition. This is the longest survival time yet reported.

Although survival time can be prolonged by use of the dialysis technique, many problems arise. One of these problems is reduced resistance of the animal to infection. A few of our animals developed a severe upper respiratory infection. A second sus-

* These studies were carried out in the laboratory of Dr. C. Riley Houck, Division of Physiology, University of Tennessee, Memphis, through whose kindness and generosity the work was made possible.

picion led us to the bacteriology problem of this paper: the possibility of bacterial peritonitis causing intussusception, adhesions, and hyperemia and hemorrhage of the gastrointestinal tract, frequently seen in the animals.

Peritoneal dialysis involves the introduction into and removal of fluid from the abdominal cavity through a needle or tubing two or three times daily. In two animals a permanent polyethylene tube was inserted through the abdominal wall; in most of the animals such tubing was inserted once daily through a trochar and retained until after the last dialysis. With such a procedure, there existed the possibility of trauma and of introduction of bacteria around or through the inserted tube or trochar. It was our desire to see if these dogs were becoming infected, to what extent, and whether or not this was an added factor in the problems described previously.

Procedure

The method plus the dialysate composition used to lavage the dogs is as described by Houck (1954). The dogs were lavaged three times daily starting in the early morning with approximately 1500 ml. of the dialysate through intra-abdominal cannula. The fluid (Table 1) was allowed to remain for

TABLE 1

Composition of Peritoneal Dialysis Fluid

	Gm/L	Na+	mEq/L Cl	HCO_3–CO_3=
NaCl	5.77	98.7	98.7	....
$CaCl_2$	0.20	....	3.6	....
$MgCl_2$	0.05	....	1.1	....
KCl	0.20	....	2.7	....
$NaHCO_3$ ...	3.00	35.8	....	35.8
Total	....	134.5	106.1	35.8

Glucose—7.5 to 30 Gm./L.
Penicillin-G, Sodium—25,000 U/L.
Streptomycin—25 mg./L.

approximately two hours, was then withdrawn by gravity tube and fresh dialysate administered again. This procedure was completed by about 3:00 p.m. After two hours the dog was fed by stomach tube and again in the evening. This system of feeding seemed to decrease early morning vomiting.

The dialysate was sterile and administered aseptically. A "before" sample was taken from each immediately before dialyzing and an "after" sample was taken as the fluid was removed from the abdomen. These samples were then plated by serial dilution technique using Trypticase-Soy agar. The

plates were incubated at 37° C. for 24–48 hours and then checked for the presence and number of bacterial colonies present. The number of organisms/ml. of dialysate was then computed.

Upon finding positive cultures immediate antibiotic therapy was started. This consisted of 200,000 units of crystalline Penicillin-G intramuscularly daily until a negative culture was obtained.

During the course of the experiment seven dogs were dialyzed, three of which acted as control dogs and were never nephrectomized. Each dog was dialyzed for approximately two weeks before and after nephrectomy.

Results

Our results may best be appraised by examining tables 2 and 3.

Summary and Conclusions. Seven dogs (3 normal and 4 nephrectomized) were peritoneally dialyzed three times daily for a period of time ranging from two weeks to one month. Samples of the dialysate were taken before and after administration and cultured bacteriologically to determine presence and number of bacteria/ml.

TABLE 2

Control Dogs

Dog No.	Control period dates	Post-nephrec. dates	Organisms per ml. before	Organisms per ml. after	Penicillin given	Comments or autopsy findings
D15	11–19		.	.	None	Control dog. Lavaged three times daily but never nephrectomized. Not sacrificed. Remained in good health.
	11–20		0	0		
	11–21		0	0		
	11–23		0	0		
	11–24		0	0		
	11–26		0	0		
	11–27		0	0		
	11–29		0	0		
	11–30		0	0		
N36	10–30		0	0	None	Control dog. Conditions same as for D15.
	11–1		0	0		
	11–2		0	0		
	11–3		0	0		
	11–4		0	0		
	11–5		0	0		
	11–6		0	0		
	11–7		0	0		
N41	11–9		0	0		Sacri. and aut. 11/18 due to severe upper respiratory infection.
	11–12		0	0		
	11–13		0	0		
	11–14		0	5		1. No adhesions.
	11–16		0	4	200,000U	2. Hyperemic GI tract.
	11–17		0	0	200,000U	3. Lung congestion and consolidation.
	11–18		0	0		

TABLE 3

Nephrectomized Dogs

Dog No.	Control period dates	Post-nephrec. dates	Organisms per ml. before	Organisms per ml. after	Penicillin given	Comments or autopsy findings
D5	10–1		0	0		Sacrificed and autopsied
N38			0	0		10/28. Findings:
	thru		0	0		1. Subendocardial hemorrhage, extensive.
			0	0		
	10–14		0	0		2. Massive adhesions.
		10–15	0	50		3. Mesenteric lymphadenopathy.
		10–16	0	40	200,000U	
		10–17	0	0	200,000U	4. Petechial hemorrhages in stomach.
		10–18	0	0		
		10–20	0	10		
		10–21	0	20	200,000U	
		10–22	0	0	200,000U	
		10–23	0	0		
		10–25	0	0		
		10–26	0	0		
		10–27	0	0		
D10	10–1		0	0		Sacrificed and autopsied
N39			0	0		10/31. Findings:
	thru		0	0		1. Subendocardial hemorrhage.
			0	0		
	10–20		0	0		2. Massive adhesions.
		10–21	0	0		3. Mesenteric lymphadenopathy.
		10–23	0	0		
		10–24	0	143		
		10–25	0	60	200,000U	4. Petechial hemorrhages throughout GI tract.
		10–26	0	0		
		10–27	0	3	200,000U	
		10–28	0	212		
		10–29	0	316		
N40	10–30		0	0		Sacrificed and autopsied
			0	TNTC		11/19. Findings:
	thru		0	TNTC	200,000U	1. Subendocardial hemorrhage.
			0	6		2. Massive adhesions: Worst seen.
	11–7		0	0		3. Mesenteric lymphadenopathy.
		11–9	0	0	200,000U	4. Entire GI tract hyperemic and with petechial hemorrhages.
		11–12	0	0		
		11–13	0	TNTC		5. Petechial hem. of gall bladder.
		11–14	0	TNTC	200,000U	6. Lung hemorrhages.
		11–15	0	500		7. Cyst of pus (size of plum) on midileum serosa. (yel.-gr.)
		11–16	0	490	200,000U	
		11–17	0	35		8. 2 lg. 4 cm. water cysts on stomach serosa.
		11–18	0	35		

TABLE 3 (Continued)

Nephrectomized Dogs

Dog No.	Control period dates	Post-nephrec. dates	Organisms per ml. before	Organisms per ml. after	Penicillin given	Comments or autopsy findings
N42	11–9		0	0	200,000U	Sacrificed and autopsied 11/30. Findings:
	thru		0	0		1. Massive adhesions.
	11–19		0	0		2. Subendocardial hemorrhage.
		11–20	0	10	200,000U	3. Mesenteric lymphadenopathy.
		11–21	0	157	200,000U	4. Entire GI tract hyperemic
		11–23	0	21		and with petechial hemorrhages.
		11–24	0	110		5. Intussusception upper ileum.
		11–26	0	100	200,000U	6. Lower jejunum encarcerated and necrotic.
		11–27	0	39	200,000U	
		11–29	0	0	200,000U	7. Multiple infarction of left ventricle.

A total of 166 samples were cultured. Eighty-three were taken before and 83 after dialysis. All 83 "before" samples were negative for bacteria. Twenty-six "after" samples were positive for bacteria ranging in number from 4/ml. to too numerous to count (TNTC).

Identification of positive cultures showed them to be *Bacillus subtilis* and *Staphylococcus aureus*. At no time was one species found without the other. This was probably a coincidental finding, however. Infections were rapidly brought under control by intramuscular administration of penicillin but tended to reappear within two to five days.

Autopsy of the four nephrectomized dogs showed the following common findings: (1) Subendocardial petechial hemorrhages—these suggest systemic bacterial infection or blood dyscrasias. (2) Petechial hemorrhages of the gastro-intestinal tract —these suggest bacterial infection or blood dyscrasias. (3) Mesenteric lymphadenopathy—suggests visceral peritonitis or enteritis which could be bacterial or traumatic. (4) Massive adhesions—suggests visceral peritoneal trauma, irritation, or peritonitis. (5) Intussusception in one dog—probably due to visceral irritation either bacterial or traumatic (probably aggravated by but not due to the dialyzing technique per se).

Two of the control dogs were maintained asymptomatic and with sterile peritoneal cavities as evidenced by continued sterile dialyzing fluids. One control had only slight contamination. This would suggest then that bacterial contamination of the peritoneal cavity is a possible etiological factor in the causation of adhesions, mesenteric lymphadenopathy, peritonitis, and intussusception.

Three of the four dogs eventually nephrectomized were maintained with sterile peritoneal cavities throughout the pre-operative control period. Only after nephrectomy did contamination appear. Nephrectomy then seems to lower the resistance of the animal to infection, the latter of which may or may not be introduced by the dialyzing technique per se.

Due to the limited number of animals in this series of experiments it is not statistically possible to say definitely that the bacterial infection is the etiological agent. This preliminary study is offered only as presumptive evidence and suggests that more work be done from a bacteriological standpoint.

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ORGANIZED ENZYME SYSTEMS*

DAVID E. GREEN

Practically all the chemical reactions which go on in the living cell do not take place to a measurable degree in a test tube unless facilitated by catalysts. Thus sugar and fats are relatively inert when exposed to oxygen under the conditions of temperature and hydrogen ion concentrations which obtain in the cell. The cell contains some thousands of organic catalysts known as enzymes each of which is uniquely adapted to carry out one special chemical reaction. The purpose of this review is not to deal with the remarkable properties of individual enzymes but rather to consider how assemblies of enzymes collaborate in implementing the overall chemical sequences by which the work of the cell is accomplished.

How is the multitude of enzymes arranged in the cell? Are we to think of the cell as a membranous bag with the enzymes floating around helter skelter in the internal fluid? The answer is that such a simple picture is far from the true state of affairs. If there is one point of which the cell chemist is certain it is that the interior of the cell is highly organized and that this organization extends down to the molecular level—to the level of the enzymes themselves.

We shall confine our attention in this review to one organized unit of the cell known as the mitochondrion. This in some cells is a cigar-shaped body about the size of a large bacterium, and in other cells a sphere of comparable volume. The mitochondrion is the physical housing for a complex of several hundreds of enzymes arranged within the unit like tiles in a mosaic—a complex known as the cyclophorase system. The mitochondrion is the meeting place of structure and function. When isolated under proper conditions from the cell the mitochondrion acts functionally in much the same way as it does *in vivo*. However, it can also be isolated in a manner which preserves form though all enzymatic activity has been lost.

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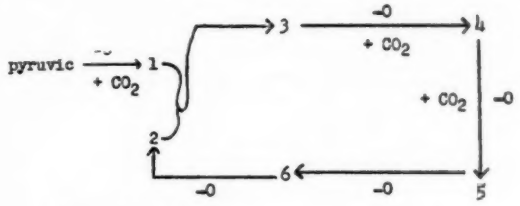
* From the Institute for Enzyme Research, University of Wisconsin, Madison. Presented at the Physiology Seminar of The University of Texas, Medical Branch, Galveston, December 2, 1953.

The millions of mitochondria within aerobic cells are the instruments by which the principal foodstuffs are burned or oxidized and by which the chemical energy thus released is transformed into other forms of energy for carrying on the work of the cell. Let us first consider the furnace function of the mitochondrion. The process by which sugar is oxidized in the animal body may be subdivided into two phases: (1) the anaerobic splitting of glucose into two molecules of pyruvic acid; and (2) the aerobic oxidation of pyruvic acid to carbon dioxide and water. The second process only goes on in the mitochondrion, and it is this process which principally provides the fuel for the furnace. Now the oxidation of pyruvic acid ($CH_3COCOOH$) to 3 molecules of CO_2 and 2 molecules of H_2O does not take place by degradation of the acid carbon atom by carbon atom. Nature has evolved a remarkable cyclical process known as the citric acid cycle by which pyruvic acid is oxidized.

The principle of the cycle shown diagrammatically in figure I is a very simple one when stripped of chemical details. Pyruvic



1—acetyl CoA.
2—oxaloacetic acid.
3—citric acid (isocitric acid, cis-aconitic acid).
4— α ketoglutaric acid.
5—succinic acid.
6—oxaloacetic acid (malic acid).

FIG. I. Diagrammatic Representation of the Citric Acid Cycle.

acid is oxidized to (1) which condenses with a partner (2) to form a compound (3) which is successively degraded by oxidation via (4), (5), and (6) eventually to form a new molecule of (2). Each turn of the cycle results in the oxidation of one molecule of pyruvic acid to carbon dioxide and water and in the regeneration of one molecule of the condensing partner. It should be noted that 5 atoms of oxygen are taken up and 3 molecules of CO_2 are liberated in each turn of the cycle.

A consequence of the cyclical nature of the citric acid cycle is that any of the intermediates of the cycle can also be oxidized to CO_2 and H_2O although more than one turn of the cycle is necessary when more than five atoms of oxygen are required. This possibility is indeed taken advantage of by the cell. Each member of the citric acid cycle may be conceived of as an entry point into the mitochondrial furnace and any substance which can give rise to one of these intermediates is also capable of being burned in the furnace. Thus fatty acids and certain amino acids fall into this category of secondary feeder substances (*cf.* Fig. II).

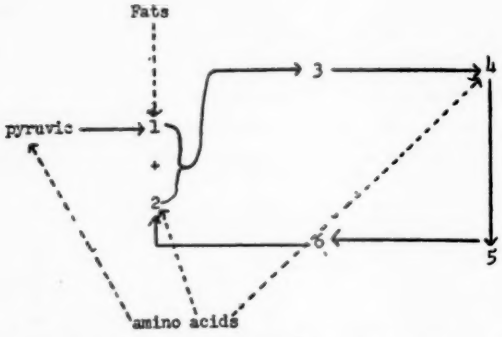


FIG. II. Secondary Feeder Substances for Citric Acid Cycle.

The chemical details of the citric acid cycle are less important for our present purposes than the events which accompany the cycle. We shall, therefore, not touch on these structural chemical details but rather concentrate on other aspects of the citric acid cycle more germane to the argument.

When any member of the citric acid cycle is oxidized by molecular oxygen two electrons are detached and transferred to oxygen. This transfer does not take place in one fell swoop but rather in stages. The electrons are passed on through each of 5 or 6 different compounds until ultimately oxygen is reached as shown in the diagram below. In order these compounds are: (1) the pyridinenucleotides which are derivatives of niacin, one of the complex of B vitamins; (2) the flavin nucleotides which are derivatives of riboflavin, another of the B vitamin complex;

and finally (3) the cytochromes whose prosthetic group is similar to the heme moiety of hemoglobin. Each of these compounds is capable of being reduced by the one before in line and oxidized by the one next in line. This spacing of the bucket brigade of electron acceptors has great bearing on the question of the utilization of oxidative energy.

The real meaning of the citric acid cycle is to be found in the energetics of the overall process. The oxidation of pyruvic acid to CO_2 and H_2O by molecular oxygen is conducted in such a way by the mitochondrial system that the bulk of the oxidative energy released is converted into another form of chemical energy with negligible heat loss. An efficiency of well over 66 per cent in an oxidative process involving molecular oxygen is unknown outside the realm of enzymes. The secret that the cell has learned is to make practically all the individual reactions in the overall process reversible. It is only when reactions are reversible that high efficiency of energy conversion can be attained.

Now let us consider the kind of process which is involved in the trapping of oxidative energy. For each atom of oxygen which is taken up during the one step oxidation of any member of the citric acid cycle, 3–4 molecules of free inorganic phosphate are converted into what we may call bound or esterified phosphate.

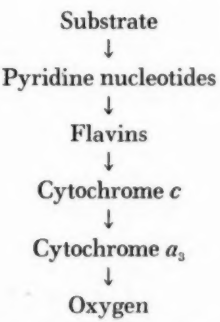


FIG. III. The Electron Transport System. The arrows indicate the direction of flow of electrons.

The conversion of phosphate from the free to the bound form requires input of energy. Thus oxidative energy is tapped as it were to provide the energy of phosphate esterification which we

may refer to as phosphate bond energy. Precisely how this energy transformation is effected is still not known in detail for each step at which phosphate is esterified though there are several markers that serve as guides. In a general way the process of oxidative phosphorylation may be represented as follows. If substance A is oxidized by B, the first product form is A_{ox}—B_{red} in which the line between the oxidized form of A (A_{ox}) and the reduced form of B (B_{red}) represents a valence bond between the pair. Now a series of substitutions take place, first for one of the pair, and then for the second. Three coenzymes are known to intervene successively in at least one such transformation. These coenzymes are in order coenzyme A (coenzyme of acyl trans-

$$
\begin{array}{c}
B_{red}—A_{ox} \\
\downarrow \\
CoA—A_{ox} \\
\downarrow \\
CoP—A_{ox} \\
\downarrow \\
CoP—P \\
\downarrow \\
AdPP—P \\
\\
AdPPP(ATP)
\end{array}
$$

fer), coenzyme P (coenzyme of acylphosphate exchange) and ADP[1] (adenosine diphosphate) the final coenzyme which on accepting inorganic phosphate (P) is converted to ATP[1] (adenosine triphosphate), the ultimate energy storage form of the cell. The principle by which oxidation and phosphorylation is linked may be put as follows. When any substance undergoes oxidation by the electron acceptor next in line a compound is formed between the oxidized substance and the reduced electron acceptor. It is possible to replace in turn each of the two members of the pair and in the end make inorganic P and ADP the two final members of the pair. Thus ATP will be formed and the new

[1] The coenzyme which serves as the final phosphate acceptor exists in three forms which differ from one another in the number of phosphate groups: Adenosine monophosphate (adenosine P) has one phosphate; adenosine diphosphate (adenosine PP or ADP) has two phosphates in a row; and adenosine triphosphate (adenosine PPP or ATP) has three phosphates.

bound phosphate husbands the energy released during the oxidation of A by B.

The mitochondrial system is so arranged that the oxidative enzymes which produce ATP and some of the synthetic enzymes which require ATP are cheek by jowl. It will be impossible to consider all the synthetic reactions in which ATP participates. However, two selected instances may illustrate the point adequately.

Fatty acids are not oxidized in the mitochondrial system except when the citric acid cycle is in operation. The connection between these two events is ATP. The operation of the citric acid cycle leads to ATP production and in turn ATP triggers the oxidation of fatty acids by a process which may be represented diagrammatically as below:

$$\begin{array}{c} \text{Adenosine PPP} + \text{CoA} \\ \downarrow \\ \text{Adenosine P} - \text{CoA} + \text{PP} \\ \downarrow \\ \text{Fatty acid} - \text{CoA} + \text{Adenosine P} \end{array}$$

The active form of the fatty acid is the fatty acyl CoA. This is produced from the free fatty acid at the expense of ATP which in the process is cleaved to adenylic acid (Adenosine P) and inorganic pyrophosphate (PP). The fatty acyl CoA can then undergo the process of β-oxidation ultimately giving rise to a member of the citric acid cycle which in turn is oxidized to CO_2 and H_2O. Thus fatty acid oxidation needs an initial lift as it were or assist which comes from ATP. Once initiated the process of fatty acid oxidation is self-perpetuating since in the final stages of fatty acid oxidation oxidative phosphorylation of the citric acid cycle members regenerates more ATP.

The first stage in fatty acid oxidation is a synthetic process, namely the synthesis of the fatty acyl CoA from the fatty acid. In other synthetic reactions the activated compound synthesized by ATP then condenses with another molecule. Thus benzoic acid after conversion to benzoyl CoA by ATP can then react with glycine to form hippuric acid. There are a variety of mechanisms by which ATP can effect the modification of one of the two substances which undergo synthesis by condensation. But it

is this modification which is the essential preliminary to all synthetic reactions. The rationale may be expressed as follows. The synthesis of AB from A and B requires energy and is improbable under physiological conditions. If A is converted to A′ then the condensation of A′ and B requires no energy and the reaction goes on smoothly. ATP foots the bill for lifting A to A′ and thereby makes possible the synthetic reaction.

The stability of the mitochondrion as a functional unit points up an intriguing dependence of function on activity. A mitochondrial suspension let us say from kidney will maintain oxidation for some hours at 38° without loss of activity. Yet the same suspension kept at 0° for the same length of time may lose a fair amount of activity particularly in absence of added metabolites. The nexus of cause and effect is something as follows. The mitochondrial unit is maintained in its proper state as long as certain coenzymes like ATP are maintained in their phosphorylated forms. During active oxidation this maintenance of state is assured. As soon as oxidation is arrested for lack of substrate or for other reasons the coenzymes break down irreversibly to states from which they cannot be retrieved by renewed oxidation. A sort of Humpty Dumpty situation sets in. Breakdown of coenzymes weakens the structural elements which keep the mitochondrial unit together and eventually disintegration of the unit ensues. We see very vividly in this instance why physiological activity is so necessary for the maintenance of a morphological unit.

Within the limits of the mitochondrial unit one can see clearly the thread which connects the many metabolic sequences—citric acid cycle with electron transport and oxidative phosphorylation, fatty acid oxidation with oxidative phosphorylation, synthetic reactions with oxidative phosphorylation and functional integrity of the mitochondrion with the operation of the citric acid cycle. This interdependence has its basis in the extraordinarily complex organization of the mitochondrial unit—an organization of which we have at present only the merest inkling. One must extrapolate beyond the mitochondrion to visualize the broader problem of organization at the enzyme level. The mitochondria are synthesized within the cell under the aegis of the hereditory units of the cell, viz., the genes. The organizational problem of putting together the hundreds of enzymes which

make up the mitochondrion is quite staggering in its dimensions. There is increasing evidence that mitochondria at least in cells like the kidney serve as instruments of physiological function. In other words the mitochondrion in addition to serving as the housing for the cyclophorase complex of enzymes also provides the mechanical means by which kidney function can be effectuated.

With the recognition of organization as a component of enzyme chemistry there has been a reconciliation of biochemistry and physiology—this time at the molecular level. The auguries for a very fruitful union now appear very good.

SMOKING HABITS OF COLLEGE STUDENTS IN TEXAS*

HELEN KIRCHOFF AND R. H. RIGDON

Few studies have been made on either the frequency or the amount of smoking in so-called healthy individuals. The earliest known estimate of the frequency of smoking in students was in 1857. At that time a student from Cambridge said, "By far the greater half of the undergraduates here smoke." (Editorial, *The Lancet*, 1857). In 1935 *Fortune Magazine* conducted a survey and found that 37 per cent of the persons interviewed smoked cigarettes; 65.5 per cent of the men under 40 years of age and 39.7 per cent of those over 40 smoked; 26.2 per cent of the women less than 40 years of age and 9.3 per cent of those over 40 smoked. Maisel in an article in *Collier's Magazine* in 1950 estimated that "more than three-fourths of all adult men and more than 40 per cent of all women smoke." Mills and Porter (1950, –52, –53) interviewed 310 white women, 20 to 29 years of age, in Columbus, Ohio and found that 39.68 per cent smoked cigarettes; 507 white women, in the same age group, were interviewed in Cincinnati and 41.35 per cent smoked cigarettes. In a group of 1153 white men over 20 years of age, 41.7 per cent smoked cigarettes, 25.6 per cent smoked a pipe, cigars or a combination of these, while 32.6 per cent were nonsmokers (Mills and Porter, 1950, –52, –53). According to Doll (1953) "some 5 per cent of men and some 50–70 per cent of women are, in England, classified as non-smokers."

During the school year 1952–53 one of us (RHR) visited eleven colleges and universities in Texas for the purpose of determining the smoking habits of the students. They were interviewed in classes and at assemblies. This study was thoroughly explained and they were asked to cooperate voluntarily by filling out the questionnaire shown in figure I.

The primary purpose of the questionnaire was to determine the number of years and the average daily amount each student

* From the Laboratory of Experimental Pathology, The University of Texas Medical Branch, Galveston. Part of a Cooperative Study with the Medical College of Virginia, Richmond. Received for publication February 27, 1954.

1–5. NAME DATE

6. SEX AND RACE: ☐ WHITE MALE ☐ COLORED MALE
☐ WHITE FEMALE ☐ COLORED FEMALE

7. AGE GROUP: ☐ 15–19 yrs. ☐ 20–24 yrs. ☐ 25–29 yrs. ☐ 30–39 yrs. ☐ 40–49 yrs.

8. CLASS:

9. Do you NOW smoke? ☐ YES
(If answer is yes, skip Q 10 to 15 and answer Q 16 to 30)

If not, have you EVER smoked? ☐ YES ☐ NO
(If answer is yes, answer Q10 to 15 only; if no, do not answer any other questions)

10. How many years did you smoke?

11. How many years since you stopped smoking?

12. Did you smoke cigarettes? ☐ YES ☐ NO

13. Did you smoke a pipe? ☐ YES ☐ NO

14. Did you smoke cigars? ☐ YES ☐ NO

15. If you smoked cigarettes, how many did you smoke daily?

16. HOW MANY YEARS HAVE YOU SMOKED?

17. Do you NOW smoke cigarettes? ☐ YES ☐ NO

18. How many years have you smoked cigarettes?

19. How many cigarettes do you NOW smoke daily?

20. How many years have you smoked the number of cigarettes in Q 19?

21. Have you ever smoked more cigarettes than the number given in Q 19? If so, what was the average daily amount?

22. Do you inhale cigarette smoke? ☐ YES ☐ NO

23. Do you NOW smoke a pipe? ☐ YES ☐ NO

24. How many years have you smoked a pipe?

25. How many pipefuls of tobacco do you NOW smoke daily?

26. How many years have you smoked the amount of pipe tobacco given in Q 25?

27. Do you NOW smoke cigars? ☐ YES ☐ NO

28. How many years have you smoked cigars?

29. How many cigars do you NOW smoke daily?

30. How many years have you smoked the number of cigars given in Q 29?

FIG. I. Smoking History Questionnaire.

smoked. In Question 16 he was asked to give the number of years he had smoked and in Questions 18, 24 and 28, the number of years he had smoked cigarettes, pipes and/or cigars. In answering Questions 19 and 20 the student gave the number of cigarettes he smoked daily and the length of time he had smoked that specific amount. The answer to Question 21 indicated whether he had ever smoked more than shown in Question 19. The number of years a student smoked and the amount of tobacco he used daily were grouped as follows:

Years	*Cigarettes*	*Pipefuls*	*Cigars*
1. Less than 1	1. Less than 5	1. 5 or less	1. 1–2
2. 1–5	2. 5–14=½ package	2. 6–9	2. 3–6
3. 6–10	3. 15–24=1 package	3. 10 or more	3. 6–10
4. 11–14	4. 25–34=1½ packages	4. Less than 1	4. Less than 1
5. 15–20	5. 34–44=2 packages		
6. More than 20	6. More than 2 packages		

The questionnaires were coded and the data transferred to IBM cards.[1]

TABLE 1

Number of Students Interviewed in Different Colleges

College	Approximate no. students in classes and assemblies	Questionnaires submitted from each group No.	Percent
1	2100	1069	50.9
2	1099	807	73.4
3	1800	1484	82.4
4	539	475	88.1
5	....	354	...
6	394	291	73.9
7	374	287	76.7
8	210	201	95.7
9	241	221	91.7
10	847	768	90.7
11	515	281	54.6
	172*	136	79.1

*Student nurses interviewed at 2 of the above schools.

[1] Acknowledgment is made to Mr. F. E. Dorsett of the Statistical Department of the Business Office, The University of Texas, Medical Branch, for his splendid cooperation and also that of his department.

Table 1 gives the number of colleges visited, the approximate number of students available for interview and the number (6,374) and per cent of students completing the questionnaire. Actually 6,582 questionnaires were turned in. However, 208 were not included because either the entire form had not been completed or the answers were facetious. The fact that we only had approximately a dozen of the latter answers indicates, in our opinion, that the students were interested and, we believe, sincere.

TABLE 2

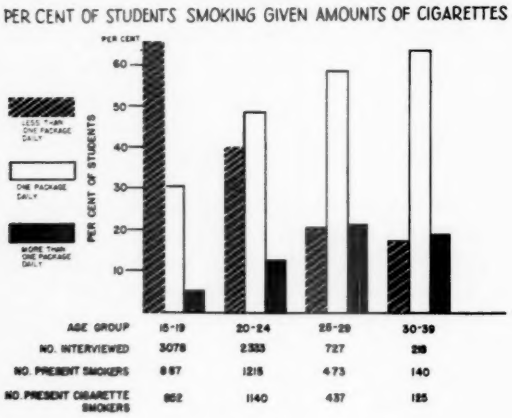
NUMBER OF STUDENTS IN STUDY
AND PER CENT OF PRESENT SMOKERS

AGE GROUP	TOTAL	%	M	%	F	%	WM	%	CM	%	WF	%	CF	%
15-19	3078	28.8	1535	38.5	1543	19.2	1127	35.7	408	46.3	763	15.6	780	22.7
20-24	2333	52.1	1797	55.8	536	39.7	1540	55.8	257	55.2	212	37.7	324	41.0
25-29	727	65.1	666	66.8	61	46.0	631	67.2	35	60.0	38	52.6	23	34.8
30-39	218	64.2	182	69.2	36	38.9	166	68.7	16	75.0	22	54.5	14	14.3
40-49	18	55.6	13	61.5	5	40.0	9	55.6	4	75.0	4	50.0	1	0.0
	6374	42.7	4193	51.8	2181	25.4	3473	52.0	720	51.0	1039	22.4	1142	28.0

In table 2 the number of students in the study and the per cent of present smokers were tabulated according to age, sex and race. In a student survey the number interviewed over 30 years of age would, of course, be small. It is interesting to note the sharp increase in the per cent of smokers from age 15 to 29. Of the entire group, 42.7 per cent were present smokers, 5.3 per cent past smokers and 52 per cent nonsmokers. Forty-eight per cent of the students were in the youngest age group, 15 to 19 years; if we omit this group, we find that 55.8 per cent smoked, 6.3 per cent had smoked and 37.9 per cent had never smoked. The difference in the per cent of smokers in the white and colored students is small but there is a wide variation in the smoking habits of the male and female. Of the entire student group, 51.8 per cent of the males and 25.4 per cent of the females smoked; however, of the students between 20 and 29 years of age, 58.7 per cent of the males and 40.4 per cent of the females were present smokers.

Graph I shows the number of students who were present cigarette smokers and the per cent of those who smoked less than one package, one package, or more than one package of cigarettes

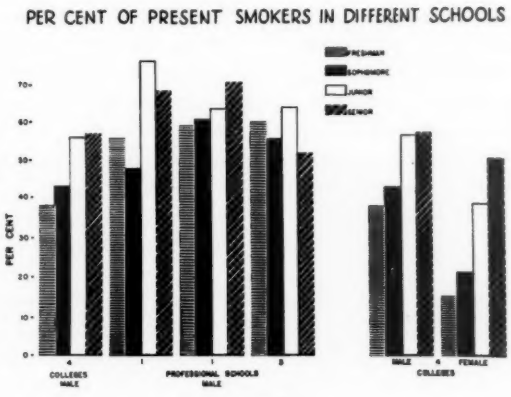
daily. Because of the small number (18) of students between 40 and 49 years of age, this group was not included in this graph. Of the cigarette smokers, 15 to 19 years of age, 30.4 per cent smoked one package of cigarettes a day. This percentage increased in the age groups 20 to 24, 25 to 29 and 30 to 39 years to 48.3, 58.3 and 63.2 per cent, respectively. Of the white males, 20 to 29 years of age, 54.2 per cent smoked cigarettes, 57.1 per cent of these smoked one package of cigarettes a day and 18.8 per cent more than one package daily. Of the group that smoked a package of cigarettes a day, 53.9 per cent had smoked that amount for 5 years or less and 40.5 per cent for 6 to 10 years. Of the group that smoked more than a package a day, 43.9 per cent had smoked for 5 years or less and 56.1 per cent for more than 5 years.



GRAPH I

Graph II shows the difference in the per cent of present smokers in the colleges and professional schools and between the male and female in the colleges. Of the male college students 37.7 per cent smoked in their first year of college, 42.5 per cent in the second year, 55.2 per cent in the third year and in the fourth college year 55.9 per cent smoked. This progressive increase in the per cent of present smokers continued into the professional schools. It is interesting to note that 37.7 per cent of the males and 14.9 per cent of the females smoked in the first year of college; how-

ever, in the senior year of college 49.0 per cent of the females and 55.9 per cent of the males smoked.



GRAPH II

The number of male students who smoked cigarettes, pipes and cigars is given in table 3. No attempt was made to separate those who smoked only cigarettes, pipes, or cigars, or those who smoked all three; the number of cigarette smokers who also smoked a pipe and the number who also smoked cigars are given. In a larger group of smokers we think the data on all types and

TABLE 3

Types of Smoking Among Male Students

	No. and age of students				
Male students	15–19	20–24	25–29	30–39	40–49
Interviewed	1535	1797	666	182	13
Present smokers	591	1002	445	126	8
Cigarettes	557	927	409	111	7
Cigarettes and pipes	84	176	65	25	1
Cigarettes and cigars	65	125	49	15	0
Pipe	108	221	87	34	2
Cigars	80	174	73	29	0

No attempt was made in this study to determine the number of combination smokers other than the number that smoked cigarettes and pipe—cigarettes and cigars.

combinations of smokers would be of considerable interest. In this selected group of students we did not consider it worthwhile.

We recognize that the accuracy of any survey is influenced by the individuals who supply the data. These students voluntarily gave their smoking histories. One of the schools included in this survey has a tradition of nonsmoking on the campus; because of this fact, our data will be on the conservative side. It is of interest to note the rapid increase in the smoking habits of the girls between their Freshman and Senior year in college. This might be referred to as "the influence of a college education on smoking." However, before such a conclusion is made, it would be necessary to determine the increase that occurs with the increase of chronological age.

The decrease in the frequency of smoking in the female over 40 years of age, as previously reported by Rigdon and Kirchoff (1953), would indicate that 20 years ago women as a group did not develop the habit of smoking. This opinion is supported by the fact that in our studies approximately 10 per cent of those that develop the habit discontinue it (Rigdon and Kirchoff, 1953). The peak of smoking is reached in the male at approximately 40 years of age. This is maintained throughout life (Rigdon and Kirchoff, 1953). From our study we have not observed that an economic factor plays a significant role in the development of this habit—it might affect the amount of tobacco used.

While smokers in these age groups could not be used as controls for smokers in cancer of the lung age groups, a study such as this would show a trend in the smoking habits of a group of young people and would be of value as to what may be expected in the future, *if* it should be proven that the increase in the frequency of cancer of the lung is the result of cigarette smoking, as has been suggested. Ochsner and associates (1952) stated that "because the carcinogenic effect of cigarette smoking does not become evident until after many years of smoking (approximately 20), it is frightening to speculate on the possible number of bronchogenic cancers that may develop as the result of the tremendous numbers of cigarettes consumed in the two decades from 1930 to 1950." These same authors (Ochsner and associates, 1952) estimate an increase from 16,450 deaths from cancer of the lung in 1948 to 47,000 in 1970. Recently Wynder *et al.* (1953) said that "in the human about 30–35 years of smoking, or ap-

proximately one-half the life span, are required for the production of bronchogenic carcinoma." From these observations it would seem that any predictions on the future death rate from cancer of the lung, resulting from cigarette smoking, would have to be based on the smoking habits of people 20 to 40 years of age today.

Summary

The smoking habits of 6,374 college and professional school students are reported. Of this group 42.7 per cent were present smokers and 52 per cent nonsmokers. Of the white males between 15 and 19 years of age 35.7 per cent smoked and of those between 20 and 29 years of age 59.1 per cent smoked. The smoking habits of the white and colored male and the white and colored female are similar, although there is a variation between the male and the female. The increase in the frequency of smoking from the Freshman year in college to the Senior year in a professional school is pointed out. The number of cigarettes smoked by the different age groups is given. In considering the frequency of smoking, it is important to establish all data for specific age groups.

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THERAPEUTIC CONSIDERATIONS IN STEROID THERAPY COMPLICATED BY EMOTIONAL DISORDERS*

JORGE Y. LARA

Various reports of emotional disorders occurring in cases receiving ACTH and/or Cortisone therapy have in the majority of instances contained theories concerning the etiology of such conditions. They have been considered both from a psychological and a physiological standpoint. Most of these reports have admitted a previous maladjustment of personality or tendency towards psychotic behavior and sometimes definite evidence of previous psychosis. Glaser states: "The previous personality pattern determines the content of the psychotic production." However the reactions of acute emotional disorder occurring as a complication in steroid therapy have to date been treated as a type of toxic psychosis: the toxic steroid substance has been withdrawn, and specific treatment not instituted unless spontaneous recovery did not occur within a reasonable period of time. In this report an attempt is made to document two particular cases which are designed to lay emphasis on the importance of the psychological elements in the production of these so-called "toxic psychoses," and also to consider some of the therapeutic implications arising from their handling.

Case History No. 1. Mrs. SGC, thirty-five year old widow, was admitted to the hospital on 5-30-52, with a complaint of loss of weight, anorexia, swelling and tenderness of the joints, erythematosus papules on her skin, with a decrease in visual acuity and a loss of hair from August, 1951. Other investigations revealed that the skin lesions had existed since 1948 and had gradually become increasingly more severe. The L.E. test was carried out and found to be strongly positive. The temperature was found to be 103.4° F., and the patient was given an initial dosage of ACTH, 40 milligrams I.M., every six hours. The fever did not diminish and Cortisone, 25 milligrams every six hours by mouth, was added and the temperature dropped to normal range within twenty-four hours. Five days after the initiation of this treatment the Cortisone was discontinued. The patient was kept on a dosage of ACTH, 10 milligrams, every twelve hours given intramuscularly. Two days later the temperature rose to 102° F and Cortisone was given orally every six hours.

* From the Department of Neuro-Psychiatry, The University of Texas, Medical Branch, Galveston. Received for publication January 29, 1954.

Improvement thereafter was gradual, the temperature became normal and the skin lesions cleared. After thirty days ACTH was discontinued; forty-eight hours later the skin lesions reappeared and the temperature rose to 103° F, and ACTH reinstituted. At this time the patient was taking 200 milligrams of Cortisone daily by mouth and 20 milligrams of ACTH I.M. daily. Signs of a depressive emotional disorder became evident at this time and on the ensuing day this depression became extreme with suicidal tendencies and also paranoid feelings that the doctor intended to kill her. One week after the onset of the psychosis the patient was transferred to the closed section of the psychopathic hospital, where she remained extremely depressed, concerned with her external appearance, stating that people did not want to look at her, and also expressing the conviction that her illness was fatal and that she wished to have her life ended as soon as possible. She made an attempt to jump out of a window and remained agitated and noisy throughout the day. She showed no evidence of sensorial disturbance at any time, but it was necessary to place her in seclusion. The Cortisone was reduced 25 milligrams daily, and the ACTH was discontinued. Cortisone was reduced in this manner, until it was discontinued fourteen days after the onset of the psychosis. During this time her temperature fluctuated between 100° F and 102° F, and she complained of joint pains. Her skin lesions became active again and her depression became more profound, as did the intensity of her paranoid ideation. Atabrine, 100 milligrams, twice daily, was started in an effort to control the symptoms of the lupus erythematosus but the psychotic picture remained unchanged, so twenty days after the discontinuation of the steroid drugs and thirty-four days after the onset of her psychosis she was begun on a course of electro-shock therapy. After the fourth treatment, a dramatic disappearance of all her depressive and paranoid symptomatology was evident. She became sociable, cheerful and optimistic regarding her physical condition and prognosis in general.

She received a total of six electro-shock treatments, the last one on 8-29-52, and she was discharged on 9-13-52, improved both from her psychosis and her skin lesions. The improvement of the skin lesions made us wonder whether or not there was some influence effected by the Atabrine. The patient was seen monthly in the outpatient department and no signs of active lupus erythematosus or psychosis were elicited when last seen on 8-19-53, having been free of symptoms for a period of eleven months.

The findings in this case were not exceptional, but they gave us reason to consider the possibility of instituting specific treatment without withdrawal of the drug since the response to specific therapy was similar to that found in functional psychiatric disorders. In the following case the findings give some clinical justification of our proposal.

Case History No. 2. Mrs. WS, thirty-seven year old white female, suffering from lupus erythematosus for ten years, was first treated with gold salts without good results. The skin lesions disappeared spontaneously after four

years, but after the patient remained asymptomatic for about three years, there was pain and stiffness appearing in the right elbow without swelling. Since that time the patient had been suffering from constant generalized pain in the joints, with occasional acute exacerbations of chills and fever. The first treatments with Pregnandiol, salicylates, sedatives, Cortisone and ACTH had been somewhat ineffective, except in the case of Cortisone, where some help had been obtained. The patient reported that she had taken "twenty boxes" of it, three or four tablets a day. The psychotic episode which brought her to our hospital appeared a few days after the restarting of one of her frequent courses of Cortisone treatment. On admission to the hospital she was found to be in extreme distress with a temperature of 104° F, which rose to 106° F on the following day. She was very depressed, without hope, weeping and expressing the conviction that nothing could be done for her. She was resistive, uncooperative, uncommunicative, unable to care for her personal hygiene or appearance and showed distinct evidence of suicidal tendencies. There was no sensorial defect. She was placed in the closed section of the hospital and treated symptomatically for her fever. The L.E. test was strongly positive and, since the temperature remained high, she was begun on daily intravenous infusions of 75 milligrams of ACTH in 1500 cc. distilled water, and seven days later the ACTH daily infusion was decreased to 50 milligrams. Five days after this new reduction, since the depressive picture became more marked, ACTH was further decreased to 25 milligrams per day. The depression did not improve and the ACTH infusion was discontinued and 60 units of ACTH gel daily was given. This did not control the fever and the gel dosage was raised to 80 units daily. During this time both patient and doctor were in a dilemma because of the severity of her physical illness and emotional disorder. It was felt that a reduction of the drug dosage would worsen her physical status to the danger point, and yet because of her nursing problem it was difficult to care for her without instituting some form of psychiatric treatment. Thus the patient was started on a regime of electro-shock treatments three times a week, and since the temperature variation was between 101° and 105° F, the ACTH gel was discontinued and Cortisone tablets, 100 milligrams every six hours by mouth, were given, which three days later were reduced to 75 milligrams every six hours. After the fourth electro-shock treatment the patient gradually became more cooperative, socialized and slept well. She was able to feed herself, to pay attention to her personal appearance, and stated that she felt much better even with her temperature fluctuations and her dosage of 75 milligrams, every six hours, of Cortisone.

This schedule was continued until the fever was controlled, and then the Cortisone was decreased to 50 milligrams, every six hours, which she took until her date of discharge. She received a total of twelve electro-shock treatments and spent the last ten days of her hospitalization on the open floor.

Discussion. Previous case reports notably by Glaser, Tourney and Gottlieb, described the appearance of symptoms occurring during treatment with ACTH and Cortisone and their occasional exaggeration after discontinuation of the drug or combined drugs.

Other reports tell of the onset of emotional disorders after the cessation of the course of the steroid drug. De Cherney reports a schizophrenic reaction occurring in a patient who had been treated with intravenous ACTH for lupus erythematosus who after considerable improvement of the skin lesions and after the discharge of the patient, she became emotionally ill on her return home. In this case the patient did not get well with electro-shock therapy but benefited from a course of deep insulin therapy. The explanation given by De Cherney for this somewhat peculiar retarded toxic reaction was "that once the organic condition which acts as a defence mechanism is alleviated or improved, the underlying heavy-laden conflicts begin to manifest themselves." This exemplifies psychopathologic etiologic theory. The majority of authors in the literature have tended toward a similar point of view. The so-called toxic effect of the drug has ben considered as a precipitant factor similar to any other emotional precipitating factor that would cause a failure of the previously successful mechanisms of defence. However, despite this general inclination it has, as we have mentioned, been the custom to treat these disorders as toxic psychosis and not as emotional disorders. The importance of this attitude is one of more than academic significance since it is generally known that the discontinuation of a specific drug inevitably exposes the patient to the dangers of the organic condition for which treatment was first instituted. In the second case reported, treatment was successfully carried out with electro-shock therapy along with the administration of the steroids, and therefore the patient was allowed the benefits which could be derived from both forms of treatment apparently without their interfering with one another. It is the feeling of the writer that further study and clinical observation is necessary to validate the hypothesis that psychiatric treatment can be given specifically in these emotional disorders complicating the steroid therapies and that possibly various methods of psychiatric treatment might be utilized.

Summary

Summary of two cases of psychosis occurring in treatment of lupus erythematosus with ACTH and Cortisone.

The first case treated with electro-shock therapy, thirty-four days after the onset of the psychosis and twenty days after the

steroid drugs were discontinued, brought about dramatic results after the fourth treatment.

The second case treated with electro-shock therapy for a similar psychotic reaction but *continued* with the dosage of ACTH and Cortisone along with the electro-shock therapy also brought about dramatic recovery.

It is suggested that the drugs act as a precipitating factor in the production of a psychosis, but once having been a precipitating factor need not necessarily be considered as a perpetuating factor.

It is hoped that with further work the validation of this hypothesis will enable physicians to continue treatment in those cases which are complicated by psychosis so as to allow them the continued benefit of ACTH and/or Cortisone therapy.

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THE LEVELS OF PERIPHERAL BLOOD LACTIC ACID IN PSYCHIATRIC PATIENTS TREATED BY EST, WITH OR WITHOUT THE USE OF A MUSCLE PARALYSANT*

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It is now a recognized fact that there is an increase of brain lactic acid (L.A.) in convulsed animals (Stone, *et al.*, 1941; Stone, 1938; Klein and Olsen, 1947; Klein and Bain, 1948). There is also evidence that the increase of L.A. corresponds chiefly to the increase of the cerebral cortical activity, being lowest in anaesthesia and sleep, higher in exercise and emotional excitement, and highest in convulsions (Stone, 1938; Stone, *et al.*, 1938; Klein and Olsen, 1947; Stone, *et al.*, 1945; Richter and Dawson, 1948). This increase of L.A. and other chemical changes in the brain tissue of convulsed animals do not depend on the chemical changes in the peripheral blood (Stone, 1938; Klein and Olsen, 1947; Stone, *et al.*, 1945; Richter and Dawson, 1948), but on the other hand, there is no conclusive evidence that the rise of L.A. (or other chemical changes) in the blood is reflected in the brain concentration of this substance and if so, whether it may influence the brain function. The question, however, of the role of L.A. as a therapeutic agent in emotional disturbances is still open. The oral or parenteral administration of L.A. gave somewhat contradictory results (Kirkegaard, 1942; Lowenbach and Greenhill, 1946). Judging from animal experiments (Richter and Dawson, 1948) one would expect rather an increase of brain L.A. in persons with so-called functional mental disturbances, especially in those having a great deal of anxiety. If the anxiety is expressed particularly in an increased muscular tension, one would also expect some increase in the blood L.A.

It was, therefore, of interest to find out whether or not there is a correlation between the amount of L.A. in blood and the therapeutic effect of electric-shock treatment. Starting with the premise that the peripheral L.A. level is due to the muscle contractions, it was decided to use succinylcholine (Anectine), a

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potent paralysant, in order to decrease the L.A. content of the blood. No reference was found in literature that there is any difference in the therapeutic results in patients treated with electric shock with or without any other paralysant (*e.g.* curare).

Methods

Nineteen physically healthy psychiatric patients were taken at random. Except patient No. 11, all the others had a diagnosis of a functional mental illness which involved various types of schizophrenia (chiefly paranoid schizophrenia) and psychotic depressive states. They were fasting before the treatment and were ambulatory in hospital wards. Each patient received a premedication of 1.3 mg. atropine given subcutaneously approximately 30 minutes before the treatment. Anectine was administered in a dose of 10 mg., except in patient No. 10 who received only 10 mg. once and later the dose was doubled. The patients received 550 mA/AC of a 1 second duration (for details of the technique, see Murray, 1953).

The samples of blood were taken from cubital veins about one minute prior to the treatment, but always before the injection of anectine. The second sample was drawn 1 to 3 minutes after an EST.

The degree of severity of the convulsions was estimated clinically by two observers and simultaneously the time of the tonic phase and total time of the convulsions were measured. The degree of severity of convulsions was arbitrarily classified into three groups: mild, moderate, and severe.

In three patients, the cerebro-spinal (C.S.F.) was also analysed for the content of L.A. Samples of C.S.F. were obtained by a lumbar puncture from fasting patients and the level of blood L.A. was estimated at the same time. Routine laboratory studies (cells, Kolmer, Lange, and proteins) of these same spinal fluids showed no abnormal findings. In one of these patients (No. 18) the C.S.F. was also examined for L.A. approximately 5 minutes after an EST on two occasions; in another one (No. 13) on one occasion one hour after the EST.

The lactic acid estimations were carried out in duplicates according to the method of Barker and Summerson (1941). Totally, 75 blood analyses for lactic acid were performed.

Results

Our findings are summarized in tables 1 and 2[1] which reveals that the fasting level of blood L.A. (before the treatment) in this series of patients (59 analyses) varies from 14.0 to 66.2 mg. per cent (average 40.1 mg. per cent). The level of L.A., however, in patients not premedicated with anectine rose after an EST to

[1] For editorial reasons the table had to be divided into two groups according to the diagnosis, however, they form a unity and no separate conclusion can be drawn from each group.

TABLE 1

Summary of Clinical Observations and the Level of Lactic Acid in Patients with Psychotic Depression

Patient No.	Sex	Age	Serial no. of treatment	L.A. before EST	L.A. after EST	Time of convulsion (sec.) Tonic phase	Time of convulsion (sec.) Total time	Degree of convulsion	Weight lbs.
1	M	47	1	66.2	172.0*	..	..	moderate	160
			2	25.5	246.0	..	..	severe	
			3	21.0	166.0*	12	40	moderate	
			4	20.5	219.0	15	50	severe	
			5	28.5	97.0*	8	37	mild	
			6	27.5	175.0	10	40	severe	
4	F	47	1	30.5	37.0*	7	30	mild	136
5	M	31	1	22.5	38.5*	11	38	mild	140
			2	24.5	151.0	8	38	moderate	
			3	30.0	36.5*	6	30	mild	
9	F	41	1	15.0	26.25*	7	29	mild	113
			2	26.5	138.5	10	42	moderate	
			3	29.0	35.0*	8	27	mild	
11	M	62	1	28.5	58.5*	8	37		154
			2	19.0	190.0	10	31		
			3	24.5	30.15*	8	39		
13	F	58	1	39.2	58.0*	10	37	mild	96
			2	34.75	105.0	9	35	severe	
			3	28.0	88.25*	8	40	mild	
			4	...	....	9	33	moderate	
			5	...	....*	7	37	mild	
			6	...	....	10	42	moderate	
			7	...	....*	9	35	mild	
			8	...	....	7	35	moderate	

*10 mg. Anectine injected.

TABLE 2

Summary of Clinical Observations and the Level of Lactic Acid in Patients with Schizophrenia

Patient No.	Sex	Age	Serial no. of treatment	L.A. before EST	L.A. after EST	Time of convulsion (sec.) Tonic phase	Time of convulsion (sec.) Total time	Degree of convulsion	Weight lbs.	L.A. in CSF before EST	L.A. in CSF after EST (time 5')
2	F	23	1	50.3	113.0*	..	..	mild	135		
			2	22.5	191.0	..	..	mild			
			3	26.5	52.0*	10	35	mild			
			4	24.0	188.5	10	45	severe			
			5	26.5	56.5*	7	37	mild			
			6	35.5	147.0	7	32	moderate			
3	F	50	1	55.0	168.0*	9	39	mild	155		
			2	53.5	200.0	8	39	severe			
			3	34.0	91.5*	7	28	mild			
			4	38.0	138.0	9	39	moderate			
			5	38.0	59.5*	7	33	mild			
			6	42.0	171.5	9	31	moderate			
			7	41.0	43.0*	8	33	mild			
6	F	28	1	38.5	54.0*	11	37	mild	136		
			2	58.0	183.5	11	35	severe			
			3	22.5	68.0*	10	38	mild			
			4	27.5	152.5	8	42	moderate			
			5	37.0	61.0*	8	35	mild			
			6	29.5	155.5	12	40	moderate			
7	F	47	1	27.0	41.0*	8	35	mild	98		
			2	52.5	173.5	12	40	severe			
			3	25.0	46.5*	9	30	mild			
			4	25.5	143.5	8	42	moderate			
8	F	49	1	23.5	66.8*	10	40	mild	121		
			2	36.0	223.5	11	36	severe			

10	M	41	1	35.76	177.65*	11	50	severe	251		
			2	28.5	198.5	8	32	severe			
			3	33.0	55.5†	8	40	moderate			
			4	37.0	135.3	8	35	severe			
			5	45.5	64.9†	9	33	mild			
12	F	47	1	...	....*	7	33	mild	112		
			2	47.5	80.0*	8	38	mild			
			3	50.0	209.3	7	37	moderate			
			4	35.0	68.5*	7	39	mild			
			5	...	....	7	38	moderate			
			6	...	....*	5	40	mild			
14	M	44	1	47.4	89.6*	9	39	mild	160		
			2	...	....	9	36	severe			
			3	...	....*	8	35	mild			
15	F	32	1	...	....*	9	39	moderate	135		
			2	...	....	8	30	severe			
			3	...	....*	8	33	mild			
			4	...	....	8	30	moderate			
16	F	31	1	...	....*	7	30	mild	192		
			2	...	....	8	30	moderate			
			3	...	....*	7	28	moderate			
			4	...	....	6	28	severe			
			5	...	....*	7	30	mild			
17	M	28	1	14.0	60.0*	10	40	mild	118		
			2	43.0	83.0	8	36	moderate			
			..	26.5	....	..	..			17.5	
18	F	23	..	...	....	..	..		105	23.5	
			1	37.5	133.5	10	41	moderate			24.5
			2	33.5	59.5*	10	39	mild			34.5
19	M	21	..	...	....	..	..		163	18.0	
			1	31.5	205.5	12	40	severe			31.0‡

*10 mg. Anectine injected.
†20 mg. Anectine.
‡L.A. in CSF 1 hour after EST.

83.0 to 246.0 mg. per cent (average 174.5 mg. per cent while in those receiving anectine it is between 26.5 and 177.6 mg. per cent, the average being 102.0 per cent). The level of blood L.A. after convulsions appeared to correspond fairly closely to the degree of severity of the convulsions.

Discussion. From our findings it follows that the increase in L.A. in the peripheral blood is due exclusively to the muscular contractions. The administration of anectine showed a relatively lower increase in L.A. level of the blood after an EST. The amount of L.A., when anectine was administered, was always much lower than that of cases without muscle paralysant (the distribution being 26.5 to 177.6 mg. per cent in the former case, and in the latter it showed 83.0 to 246.0 mg. per cent), but it was still much higher than the L.A. level before the electric shock was applied (14.0 to 66.2 mg. per cent). This phenomenon was due to the fact that all patients, irrespective of body weight, except No. 10, received the same amounts of anectine (10 mg.); therefore, the paralyzing effect varied as seen from tables 1 to 2. In the case of patient No. 10, who weighed 251 lbs. and who received 10 mg. anectine in his first treatment, the muscular contractions were very slightly reduced and the L.A. in blood was 177.6 mg. per cent). In the next two treatments with anectine he received a double dose of the muscle paralysant and the L A. dropped considerably, to 55.5 and 64.9 mg. per cent, respectively.

In the majority of our patients, the pre-EST levels of blood L.A. were slightly higher at the beginning of a course of treatment. Although one cannot draw any definite conclusions from this, it seems likely that this may be due to a greater amount of anxiety (probably expressed in increased muscular tension) in these patients at the beginning of treatment and it corresponds perhaps to findings of Richter and Dawson (1948) in their experiments on animals.

The duration of convulsions did not appear to be related either to the use of anectine or the levels of L.A. found before and after an EST.

No correlation between the level of peripheral blood L.A. and the therapeutical results of an electric shock could be established. We tend to agree, therefore, with Kirkegaard (1942) that the administration of lactate to the patient seems not to have a thera-

peutic effect as claimed by Lowenbach and Greenhill (1946). It has to be stressed that our findings do not shed any light upon the relations between increased L.A. in the brain and the recovery of the patient. In our limited number of estimations of L.A. in the C.S.F. as compared to the level of the blood, we could not find any parallel increase of L.A. in the C.S.F. after convulsions, which points towards the fact that the L.A. does not penetrate the blood-C.S.F. barrier. In conclusion, it has to be stressed again that the administration of L.A. to the patient cannot be considered as a substitute for an EST.

Summary. Levels of L.A. in peripheral blood were estimated in 19 patients before and after EST. In approximately one-half of the electro-shock treatments a premedication with a muscle paralysant anectine (succinylcholine) was used.

After EST, the levels of L.A. increased to approximately twice the pre-EST level when anectine was used, while without its use the increase was about five times as great.

The therapeutic results of electroconvulsive therapy cannot be correlated with the levels of blood L.A. after the treatment.

The levels of blood L.A. appear to correspond closely to the degree of muscular contractions resulting from an electrical shock.

The duration of the convulsions in EST does not seem to have any significant effect on the blood L.A. levels.

After an EST, no increase of C.S.F. level of L.A. above the pre-EST blood L.A. level was found in two patients.

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THE PHARMACOLOGICAL BACKGROUND OF THE TREATMENT OF BARBITURIC ACID POISONING

With a Brief Survey of the Treatment of This Poisoning in Denmark and Its Results*

Knud O. Møller†

Barbituric acid poisoning[1] is so common that it is very important to have an effective treatment against this poisoning. The most frequent treatment through many years and that still employed in most countries lays the *main stress* on initial *gastric lavage*, which is regarded as necessary, as well as on stimulation of the central nervous system by analeptics. This appears from a number of papers published within recent years amongst others in American journals. The discussions in these papers are chiefly concerned with the question whether picrotoxin is still to be regarded as the most suitable drug against barbituric acid poisoning, or whether amphetamine, nikethamide, or metrazol are preferable. In case of insufficient respiration oxygen therapy with maintenance of free air passages, as well as fluid therapy against dehydration have within the last few years been recommended in some papers. There are, of course, given antibiotics against pneumonia, and other symptomatic treatment.

The results of this therapy are, however, by no means good, the mortality being in many statistics between 20 and 30 per cent. Partly on this account, and partly because the number of severe barbituric acid poisonings is unusually large in Denmark in proportion to the population, we already before the second world war commenced an experimental investigation in our Department with the object of finding a rational treatment against acute poisonings.

This led to a close collaboration with The Department of Psychiatry at the Bispebjerg Hospital, Copenhagen (Head: Dr. Carl

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[1] It is usually the barbituric *acids* that are taken by mouth, and in the blood (pH 7.3) they are found chiefly as free acids. Therefore the expression "Barbiturate poisoning" is incorrect.

Clemmesen). About 1947 they began to change the treatment hitherto employed in this Department. *Experimental* as well as *clinical* observations constitute the basis of the therapy that is now universally employed in Denmark and of which I shall give a brief account.

During the years 1945 and 1946, before the therapy had been changed, there occurred about 1,500 cases of severe barbituric acid poisoning annually in the whole country. Of these, about 370 each year proved fatal, that is, a mortality rate of about 25 per cent (Fig. I). In 1951 the number of cases of barbituric acid

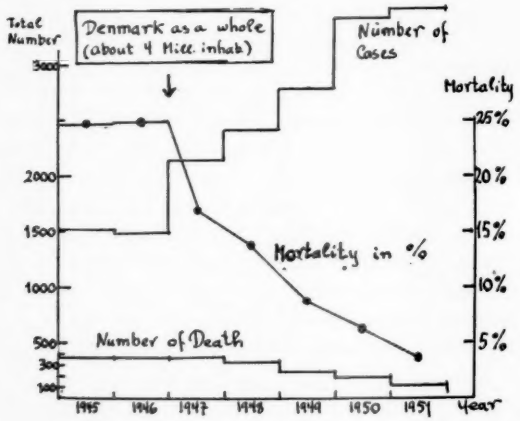

FIG. I. Total cases of barbituric acid poisoning in Denmark in the years 1945–1951 (ordinate to the left) and the mortality rate (ordinate to the right). Lower curve: Total number of deaths.

In the beginning of the year 1947 the new treatment was started in Copenhagen, but not in the final adopted form; in 1951 the treatment as described here was nearly universally adopted in the whole country.

poisoning in the whole country had risen to 3,000, but only 111, or 3.7 per cent, of these died. In Copenhagen (Fig. H), where *all cases are now treated in the stated hospital, 1,276 thoroughly observed patients were treated in 1951, of whom only 1.6 per cent (21 patients) died.* Most of the 21 patients who died were dying on admission and died a few hours later.

Thanks to the thorough clinical investigations carried out by Dr. Clemmesen and his collaborators, on the basis of the experi-

mental works, they can now, in the stated Copenhagen hospital department, where the treatment of all poisonings are concentrated, cure practically any person admitted with acute poison-

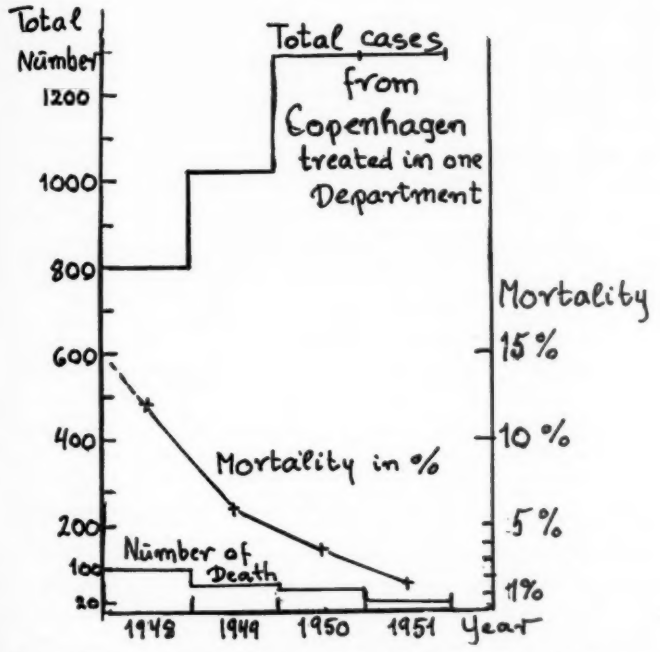

FIG. II. The cases from the Bispebjerg Hospital in Copenhagen in the years 1948–1951. See text to figure I.

ing by barbituric acids, morphine, or other narcotics, except the persons that are dying on admission.

Experimental and Clinico-Experimental Investigations

Activated Charcoal. Activated charcoal (carbo medicinalis) adsorb many poisons effectively, thereby slowing down the absorption rate of the poisons sufficiently to prevent toxic effects. Experiments made in our Department (Harrestrup, Andersen, 1946, 1948[2]) showed that 1 g. charcoal can adsorb maximally

[2] The experiments had been carried out in 1939–1940.

700 to 1,200 mg. of the non-ionised barbituric acids in aqueous solution. At pH values lower than 7.5 to 7.0 the barbituric acids are found predominantly as non-ionised free acids (of the ionised barbiturates only 300 to 400 mg. are adsorbed per g. charcoal).

TABLE 1

Amount of Substance Adsorbed by 1 g. Carbo med. Merck

Adsorbendum	Maximal adsorption in mg.
Mercuric chloride	1800
Sulfanilamide	1000
Strychnine nitrate	950
Morphine hydrochloride	800
Atropine sulfate	700
Nicotine	700
Barbital ("Veronal")	700
Barbital-sodium ("Medinal")	150
Phenobarbital-sodium ("Luminal")	300–350
Aprobarbital	
Dial-sodium ("Dial")	
Evipal-sodium	
Phanodorn-calcium	
Salicylic acid	550
Phenol	400
Alcohol	300
Potassium cyanide	35

In normal gastric and intestinal contents, where the barbituric acids occur chiefly as free acids, somewhat smaller quantities are adsorbed than those just mentioned, but still so much that charcoal might *a priori* be regarded as a suitable antidote against barbituric acid poisoning, just as it is efficient against numerous other poisonings. Experiments on rabbits and dogs also showed that charcoal reduced very considerably the anaesthetising action of barbituric acids administered by mouth (Harrestrup Andersen, 1948). For reasons to be mentioned later we find it inadvisable, however, to use activated charcoal as antidote against barbituric acid poisoning in cases where the reflexes are abolished.

Gastric Lavage. Gastric lavage has for more than 50 years been regarded as an indispensable link in the treatment of any form of acute poisoning. A thorough review of the literature showed that not a single experimental work was available that could give answers to the following questions: How much of a

poison taken by mouth is really removed by gastric lavage when this is performed within the first few hours of intake? How much fluid is required for the lavage?

These questions have therefore been studied in the previously mentioned hospital department in collaboration with our laboratory. More than 100 persons admitted unconscious after intake of soporifics were immediately submitted to gastric lavage with a total of 10 litres of water, divided into five accurately measured portions collected separately. The amount of poison in the wash-water was then determined by an exact chemical method and identified by micro-melting-point determination (Harstad, Møller, and Simesen, 1942).

The main results of examination of 72 barbiturate-poisoned persons appear from table 2. In 62 per cent of the cases less than

TABLE 2

Barbituric Acid Found in the Fluid Collected From Gastric Lavage with 10 Litres of Water in 72 Cases of Poisoning with Barbituric Acids

Barbituric acid found in fluid from gastric lavage	Number of cases total		in percent	
0	38	45	= 62	86%
0–0.01 g	7			
0.01–0.1 g	17		= 24	
0.1–0.26 g	8		= 11	
> 0.5 g	2		= 3	
Total:	72		= 100%	

10 mg. barbituric acid were removed by the lavage, and in 86 per cent less than 100 mg., that is, a quite insignificant amount. In one case the lavage was performed 30 minutes after the patient concerned had taken 6.0 g. phenobarbital. No more than 210 mg., or 3 per cent, of the quantity ingested could be demonstrated in the wash-water. That the poisoning was of a severe nature in all the cases investigated appears from the fact that the mortality rate was 23 per cent. The two patients in whose wash-water there was found more than 0.5 g. died. The gastric lavage had, in other words, had no curative effect in these cases either. (These investigations were performed before the new treatment was introduced.)

In experiments on big dogs, whose stomach had been ligated at the pylorus so that fluid could not pass from the stomach into the intestine, it appeared that by washing with 1 or 2 litres of water it was possible to remove 75 to 80 per cent of the barbituric acid previously introduced into the stomach. The absence of barbituric acids in the wash-water therefore cannot be due to the lavage as such being unable to remove poisons from the stomach.

We have explained the results achieved as follows: Persons who intend to commit suicide rarely eat before they take the poison. Our examinations of numerous samples of gastric contents from other cases of poisonings have proved this. The barbituric acids are taken on an empty stomach and as a rule washed down with abundant fluid. *The poison is therefore immediately carried into the intestine, thus having already passed the stomach by the time the patient is received for treatment.*

In our experiments we washed with altogether 10 litres of water, but only on an average about 7 litres could be withdrawn again. Thus, about 3 litres of the wash-water passed into the intestine. For further illustration of this fact we treated a small series of patients with severe barbituric acid poisoning by washing the stomach with a suspension of barium sulphate in water, while at the same time we took X-ray photos.

In all these cases a considerable proportion of the fluid introduced into the stomach was found promptly to leave this, so that on concluded lavage the small intestine was seen to contain contrast medium, in some instances as far as the coecum. Already about 1 minute after introduction of 1 litre of the contrast fluid in the stomach a very considerable proportion of it was found in the intestine. We must therefore expect that, in the relatively few cases *where poison is still present in the stomach, this is immediately conveyed into the intestine with the first portion of wash-fluid, so that the absorption is accelerated by the gastric lavage and the condition is made worse for the patient.* In barbituric acid poisoning the pyloric sphincter very soon becomes subject to severe paralysis. This contributes to the prompt passage of fluid into the intestine. Through other investigations we have shown that in barbiturate-poisoned humans there is as a rule found severe atony of the intestine, so that laxatives, often employed in the classical treatment, have no effect.

In the stated clinico-experimental investigations we concluded the gastric lavage with introduction of activated *charcoal*. In the patients who died we found numerous charcoal particles in the aleveoli of the lungs. This showed that a fairly large quantity of the charcoal suspension is aspirated into the lungs, a fact which essentially reduces the patient's chances of recovery and increases the risk of pneumonia.

Our Experiments Thus Gave the Following Results. That gastric lavage only very occasionally removes some of the orally taken poison, that gastric lavage furthers the passage of poison from the stomach to the intestine, thus accelerating the absorption, and that fluid introduced into the stomach of an unconscious person with abolished pharyngeal and laryngeal reflexes very often is aspirated into the lungs. Gastric lavage therefore, in our opinion, involves a great danger to unconscious persons without yielding any benefit. *It must accordingly be regarded as contraindicated for patients suffering from barbituric acid poisoning or other narcotic poisonings.*

Patients who have taken the narcotic poison not more than 4 or 5 hours before the commencement of treatment ought to have a stomach tube introduced and the *stomach emptied by aspiration*, but only if the pharyngeal and laryngeal reflexes are in some degree preserved.

Even though the stated results concerning gastric lavage have been described in detail in the *J.A.M.A.* (Editorial, 1947), we have found only few articles within more recent literature in which a warning is pronounced against gastric lavage to unconscious patients.

Which Is the Cause of Death of Barbituric Acid-Poisoned Patients? Until a few years ago this question could not be definitely answered. Death has been supposed to be due to depression, or possibly *paralysis of the respiration*, among others. Depressed respiration can easily be produced in animal experiments by giving sufficiently large doses of barbituric acid. Such can also be seen in man after intake of excessively large doses. Death then occurs within relatively few hours. This is, however, a very rare occurrence. As a rule the patients die 2 or 3 days after the intake of the poison. (Death following morphine poisoning is nearly always due to respiratory paralysis.)

Aspiration pneumonia is a frequent phenomenon and has by numerous writers been regarded as the cause of both death and the hyperthermia often observed. When we first started our investigations I was present at numerous post-mortem examinations of patients dead from barbituric acid poisoning. In the discussions between pathologists and pharmacologists we always agreed that the pathological changes in the lungs seemed far too slight and too limited to account for the occurrence of death.

This fact, among others, prompted A. Kirkegaard, from the Bispebjorg Hospital—at the suggestion by G. C. Brun in our Department—to make some experiments on rats in 1946 (published in English in 1952). He poisoned the animals with aprobarbital (allylisopropyl barbituric acid), and then treated them in different ways (Table 3). Arterioles and capillaries in the

TABLE 3

Survey of Experiments on Rats Poisoned by Allylisopropylbarbituric Acid and Treated in Different Ways (Kirkegaard, 1952)

Ap. = aprobarbital = allylisopropylbarbituric acid.
art. resp. = artificial respiration.

Treatment	Time between administration of aprobarbital and the death
Ap alone	¾ hour
Ap + art. resp.	4 hours
Ap + art. resp. + serum	10–12 hours
Ap + serum	¾ hour

splanchnic area were submitted to microscopy *in vivo*, so that the blood circulation in this area could be directly observed. Further, the haemoglobin concentration was measured. The rats were given a barbituric acid dose that without treatment caused death from respiratory paralysis within 40 to 45 minutes.

If the rats were treated by *artificial respiration*, death occurred after on an average 4 hours. Examinations of these rats showed that the haemoglobin concentration began to rise between 1 and 2 hours after the administration of barbituric acid, finally to reach high values. In other words, a pronounced haemoconcentration occurred. Direct microscopy revealed a pronounced fall in the rate of blood flow in the mesenteric vessels. Thus, a typical picture of severe secondary circulatory shock was demonstrated. Various control experiments showed that this shock was not due to operation, artificial respiration, or other experimental factors, but exclusively to the aprobarbital.

By adding rat serum, rat blood, horse serum, or dextran after the shock had begun to develop in the rats submitted to artificial respiration the animals could be kept alive with a normal circulation as long as the individual experiments were continued (up to 10 or 11 hours). (The rats lay immersed in Tyrode's solution of body temperature for the sake of the microscopy of the vessels.)

If treated with serum alone the rats died after on an average 40 to 45 minutes from respiratory paralysis, just like the non-treated rats, that is, at a point of time when the circulatory shock had not yet manifested itself.

Simultaneously with the animal experiments Kirkegaard (1946) made thorough clinical investigations on persons admitted to the Bispebjerg Hospital with severe barbituric acid poisoning. It appears from these that respiratory paralysis hardly ever was the direct cause of death. The respiration may remain fairly normal far into the state of poisoning, and if it tends to become depressed, it is nearly always possible to maintain sufficient respiration with *moderate* doses of analeptics. On the other hand, Kirkegaard found that all the patients developed the signs and symptoms characteristic of secondary circulatory shock, namely haemoconcentration, fall in blood pressure and diminution of the blood pressure amplitude, small, rapid pulse, peripheral cyanosis and coolness, reduced turgor, gaunt face, and changes in the bicarbonate and chloride concentrations of the blood of the same relative magnitude as in shock.

The mortality in this group of patients was reduced substantially by Kirkegaard by treating the patients with infusion of serum or dextran as well as by fluid therapy, like in other forms of shock.

These results prompted Lous, *et al.* (1949), and Nilsson (1951), to further clinical studies in the University Hospital (Copenhagen) and the Bispebjerg Hospital, respectively. They showed that even better results could be obtained if continuous oxygen therapy could be carried through side by side with the treatment just mentioned, with special emphasis on keeping the air passages free by means of the methods employed in modern anaesthesiology.

A characteristic and frequent sign in severe barbituric acid poisoning is an *excessive temperature rise* relatively late in the

course of the poisoning, during which the patient often dies. The cause of this *hyperthermia* has so far been unknown. It cannot be explained as due to a possibly occurring pneumonia. Nilsson, in his monograph from 1951, has advanced the hypothesis that an essential cause of the hyperthermia is the treatment hitherto employed with frequent and very large doses of analeptics. After they had *abandoned the treatment with analeptics in massive doses* in the Bispebjerg Hospital these alarming and often fatal elevations of the body temperature practically ceased to occur (Clemmesen, 1953, personal communication). As another possible cause of the hyperthermia I wish to point at the severe anoxia, which was often observed during the former treatment and which now seldom occurs.

Through these investigations we have attained to the treatment of barbituric acid poisoning that is now most frequently employed in Scandinavia, and which has reduced the death rate from this poisoning from more than 20 to less than 2 per cent.

Brief Survey of the Treatment of Barbituric Acid Poisoning Employed in Denmark.[3] The space allows me no more than a brief survey of the treatment. For details I must refer to the previously mentioned publications.

The patients are admitted to a *special department* of the Hospital, and treatment is started immediately on admission by specially trained doctors and nurses. *The patients are watched over*, the general condition, circulation, fluid and electrolyte balance, respiration, etc., being regularly controlled. It is, for instance, very important to recognize the first slight signs of secondary circulatory shock (haemoconcentration, etc.).

On admission the *gastric contents are aspirated*, if no more than 4 or 5 hours have elapsed since the intake of the poison, and the pharyngeal and laryngeal reflexes are intact. Gastric lavage is of course never performed.

The patient is placed in a slight *Trendelenburg position* (about 5°) during the first few days to prevent aspiration of gastric contents. Heels and knees are packed with cotton wool to prevent bed sores.

A hollow tongue depressor is, as soon as possible, inserted, through which *ozygen* is administered in a continuous current.

[3] Dr. Clemmesen has given me a manuscript in which the treatment is described in detail.

The patients are turned over every two hours. At the same time intense slapping of the chest wall and *suction of secretion from the air passages are performed.*

Penicillin or the like is given prophylactically. The *electrolyte* (including potassium) and *fluid balance* is, as far as possible, maintained in the usual way.

It is important to arrest *shock* before it has become fully developed. At the first sign of shock a solution of *dry serum* or dextran, or possibly blood transfusion is given. Often half a litre suffices, but in severe cases 2 or even 3 litres may be required, but care must be taken to avoid pulmonary oedema. A *concentrated solution of dry serum*[4] is particularly efficient against threatening pulmonary oedema or if there are other reasons for hesitating before administering large amounts of fluids.

The air passages must, as stated, be kept free by frequent aspiration from the pharynx and the larynx, and, if necessary, from the trachea after intubation. The tracheal tube must be changed daily and should not be left for more than 24 to 48 hours, unless absolutely necessary.

The continuous oxygen therapy must, of course, be carried on as long as there are the slightest signs of shock.

In case of depressed respiration *analeptics in moderate doses* are given. Picrotoxin is never used. *Amphetamine* is given in a single, relatively large dose, 25 mg., intramuscularly, as this, in addition to stimulating the central nervous system, may be helpful in producing a more ample flow of blood in collapsed veins. This facilitates the venous puncture in a shocked patient.

Gradually as oxygen therapy has become systematically carried through, *pulmonary oedema* has been reduced to an uncommon complication. Its occurrence indicates intubation with aspiration and prompt intravenous injection of a hypertonic solution of glucose (100 ml. of a 50 per cent solution, for instance) and in some cases a concentrated solution of dry serum. As a rule, pulmonary oedema rapidly yields to these measures.

Heart failure is a rare complication, except in patients with a pre-existing heart disease. If present, strophanthin or cedilanide may be administered. Caution should be exercised in administering fluids.

[4] The dry serum is solved in half of the volume of liquid as normally used.

Hypothermia—often present on admission—is easily relieved by the usual measures. In case of *hyperthermia*, which is now a rare complication, the cause of the fever must be traced. Special attention must here be given to the possibility of atelectasis and of drug fever. The usual methods are employed to lower the raised body temperature.

Finally, a few words about *morphine poisoning*.

While barbituric acid poisoning is characterised by a secondary circulatory shock and a fairly well-preserved respiratory function, morphine poisoning is, on the contrary, characterised by pronounced impairment of the respiratory function and a primarily intact circulatory function. *If the oxygen tension of the arterial blood can be maintained by suitable treatment, the blood pressure and pulse will usually remain normal through many hours, even if all spontaneous respiration has ceased.* Treatment of the circulation is unnecessary.

The treatment of morphine poisoning—beside injection of nalophine (N-allylnormorphine)—therefore consists in the first instance in artificial ventilation of the lungs with oxygen, by means of a suitable apparatus for anaesthesia or the respirators used in the treatment of poliomyelitis. If this is omitted, anoxia will often occur, which again leads to secondary circulatory shock.

As I have mentioned, we can now cure practically all barbituric acid poisoned patients by the method of treatment elaborated in Denmark, and now used in all the Scandinavian countries. A comprehensive statistical report based on a Danish series of patients showed (Hove, 1953) that only about 5 per cent of those who had attempted suicide by intake of barbituric acid and had been saved from death tried once more to commit suicide. The remaining 95 per cent were all happy to have been saved. The doctor naturally feels a professional satisfaction in each successful treatment. The stated statistical report shows, however, that from a humane point of view we also have good reason to be satisfied that by this treatment we are able to help

so many persons through the consequences of such a most often rash and desperate act as that of attempting suicide.

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THERMAL STRESS AND TISSUE CULTURE

III. Gey's Balanced Salt Solution Versus Protein Containing Fluids as Freezing Media*

J. Nakai,† C. M. Pomerat, and P. F. Gilliland

All experiments which were reported in the first and second manuscripts published in the *Texas Reports on Biology and Medicine* on *Thermal Stress and Tissue Culture* (I. The effect of rapid freezing and thawing on chick embryonic epidermis, skeletal muscle and spindle cells (11: 307–332, 1953); II. The effect of rapid freezing and thawing on human newborn and adult skin (11: 333–346, 1953)) involved freezing in Gey's balanced salt solution. The justification for this procedure lay in the fact that in our laboratory tissue fragments prepared for culture are customarily cut and washed in this solution. Moreover, in several cinematographic studies no serious disturbance in membrane activity has been recorded with the use of phase contrast time-lapse cinematography when various species of human cells have been exposed to at least 24 hours' perfusion with Gey's balanced salt solution. It must also be recognized that the actual period of freezing generally involved residence in salt solution for only a few minutes. However, since frostbite in man occurs under conditions in which the cells are bathed by tissue fluids it was felt desirable to check whether resistance to cold injury as defined with tissue culture methods might reveal important differences when explants were frozen in some protein containing medium such as blood serum or ascitic fluid in contrast to a physiological salt solution.

Provisional experiments showed the type of minor variations in results typically associated with these studies. At the outset it was our impression that freezing in serum might prove less harmful than freezing in Gey's. Slight differences in the age of the explants and in the nature of the nutrient media as well as

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variations in the time required to achieve a given drop and recovery from the desired temperature could be seen to account for some differences in the quantity of surviving epithelial muscle and spindle cells.

Figure I illustrates the thermal records on experiments 104 to 112 in which 12-day chick skin and leg muscle were frozen in

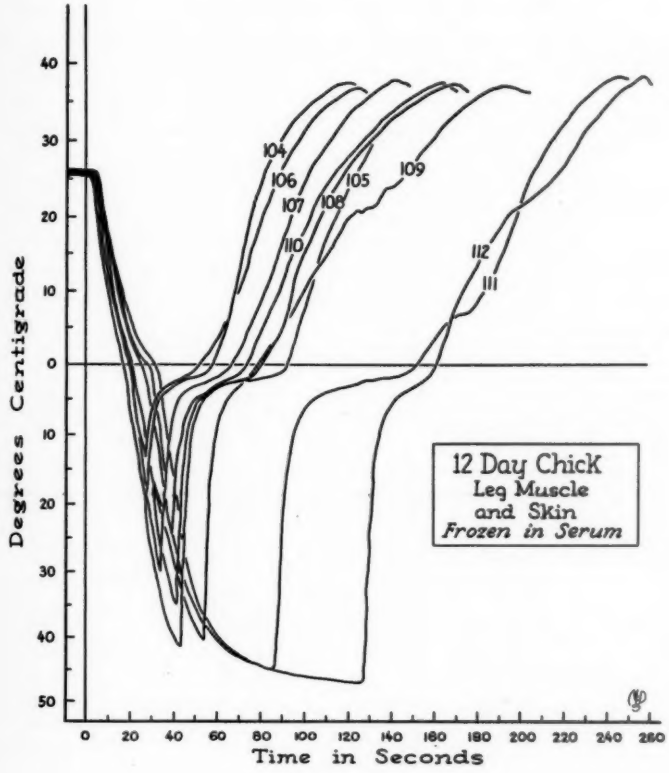


FIGURE I

fowl serum. The results obtained following the cultivation of these fragments are given in table 1.

Chick tissues frozen in Gey's balanced salt solution were found by Pomerat and Lewis (1953, I.) to show survival of muscle

fibers after exposure to approximately —20.0° C., epithelium after —30.0° C., and spindle cells after —40.0° C. It would seem therefore that the Gey's solution had been somewhat less effective in protecting against cold injury than the fowl serum.

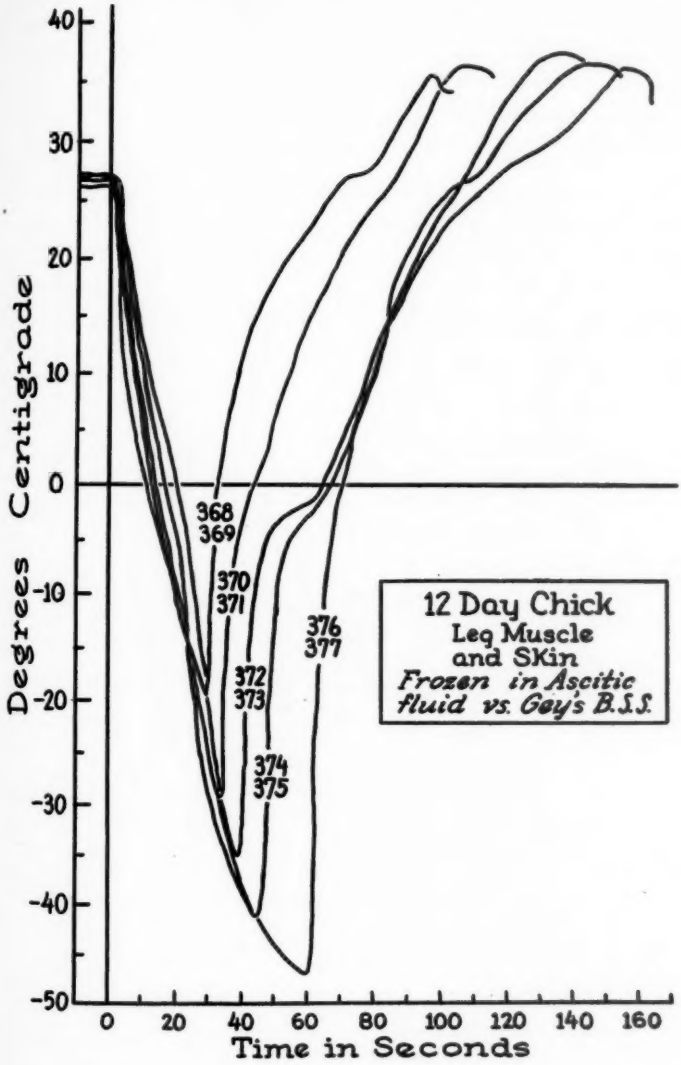
TABLE 1

Results Obtained From 12-day Chick Embryo Explants Frozen in Fowl Serum

Experiment No.	Skin		Leg Muscle	
CONTROL— unfrozen	Epithelium	16/20*	Muscle	18/20
	Spindles	17/20	Spindles	20/20
	No outgrowth	3/20		
Experiment 104	Epithelium	14/20	Muscle	14/20
−18.8° C. in 28 secs.	Spindles	14/20	Spindles	20/20
	No outgrowth	6/20		
Experiment 105	Epithelium	8/20	Muscle	10/20
−24.3° C. in 38 secs.	Spindles	8/20	Spindles	19/20
	No outgrowth	12/20	No outgrowth	1/20
Experiment 106	Epithelium	10/20	Muscle	10/20
−13.4° C. in 28 secs.	Spindles	20/20	Spindles	20/20
Experiment 107	Epithelium	9/20	Muscle	11/20
−30.0° C. in 35 secs.	Spindles	10/20	Spindles	20/20
	No outgrowth	10/20		
Experiment 108	Epithelium	6/20	Muscle	13/20
−35.0° C. in 43 secs.	Spindles	6/20	Spindles	18/20
	No outgrowth	14/20	No outgrowth	2/20
Experiment 109	Epithelium	2/20	Muscle	6/20
−40.5° C. in 53 secs.	Spindles	2/20	Spindles	15/20
	No outgrowth	18/20	No outgrowth	5/20
Experiment 110	Epithelium	5/20	Muscle	1/20
−41.3° C. in 44 secs.	Spindles	8/20	Spindles	16/20
	No outgrowth	12/20	No outgrowth	4/20
Experiment 111	Epithelium	1/20	Muscle	5/20
−45.3° C. in 87 secs.	Spindles	1/20	Spindles	16/20
	No outgrowth	19/20	No outgrowth	4/20
Experiment 112	Epithelium	5/19	Muscle	5/20
−47.0° C. in 128 secs.	Spindles	5/19	Spindles	13/20
	No outgrowth	14/19	No outgrowth	7/20

*Out of 20 explants 16 showed epithelial outgrowth.

In order to achieve more homogeneity of experimental conditions, it was decided to freeze tissues in both types of media simultaneously. For the various temperatures to be studied, a pair of thin-walled conical centrifuge tubes containing 2 ml. for each of the two media and identical tissue content were employed.


Degrees Centigrade
40
30
20
10
0
-10
-20
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0
20
40
60
80
100
120
140
160
Time in Seconds
368
369
370
371
372
373
374
375
376
377
12 Day Chick
Leg Muscle
and Skin
Frozen in Ascitic
fluid vs. Gey's B.S.S.

FIGURE II

TABLE 2

Results Obtained From 12-day Chick Embryo Explants

Expt. No.	FROZEN IN ASCITIC FLUID Skin		Leg Muscle		Expt. No.	FROZEN IN GEY'S BALANCED SALT SOLUTION Skin		Leg Muscle	
CONTROL— unfrozen	Epithelium	8/11	Muscle	14/17	CONTROL— unfrozen	Epithelium	14/17	Muscle	18/20
	Spindles	11/11	Spindles	17/17		Spindles	17/17	Spindles	20/20
Expt. 368 –19.3° C. in 30 secs.	Epithelium	0/16	Muscle	7/13	Expt. 369 –19.3° C. in 30 secs.	Epithelium	2/17	Muscle	7/14
	Spindles	6/16	Spindles	11/13		Spindles	17/17	Spindles	11/14
	No outgrowth	10/16	No outgrowth	2/13				No outgrowth	4/14
Expt. 370 –29.3° C. in 34 secs.	Epithelium	0/20	Muscle	1/20	Expt. 371 –29.3° C. in 34 secs.	Epithelium	0/20	Muscle	0/19
	Spindles	1/20	Spindles	13/20		Spindles	12/20	Spindles	19/19
	No outgrowth	19/20	No outgrowth	7/20		No outgrowth	8/20		
Expt. 372 –35.2° C. in 40 secs.	Epithelium	0/20	Muscle	0/20	Expt. 373 –35.2° C. in 40 secs.	Epithelium	0/10	Muscle	1/20
	Spindles	6/20	Spindles	5/20		Spindles	1/10	Spindles	10/20
	No outgrowth	14/20	No outgrowth	15/20		No outgrowth	9/10	No outgrowth	10/20
Expt. 374 –41.0° C. in 44 secs.	Epithelium	0/16	Muscle	0/15	Expt. 375 –41.0° C. in 44 secs.	Epithelium	0/20	Muscle	1/16
	Spindles	1/16	Spindles	1/15		Spindles	4/20	Spindles	3/16
	No outgrowth	15/16	No outgrowth	14/15		No outgrowth	16/20	No outgrowth	13/16
Expt. 376 –47.0° C. in 60 secs.	No outgrowth	20/20	No outgrowth	20/20	Expt. 377 –47.0° C. in 60 secs.	Epithelium	0/30	No outgrowth	20/20
						Spindles	5/30		
						No outgrowth	25/30		

Having cut a sufficient quantity of chick skin and muscle from 12-day embryos to provide 20 tissue fragments for each of both tubes, it was hoped that minor variations in tissue sampling would be reduced. Thus, 80 pieces of tissue were frozen simultaneously for each temperature (Fig. II) and results for a typical series of experiments obtained after 6 days' incubation are represented in table 2.

In view of the variability of results, a final group of check experiments was performed using homogeneous tissue samples and

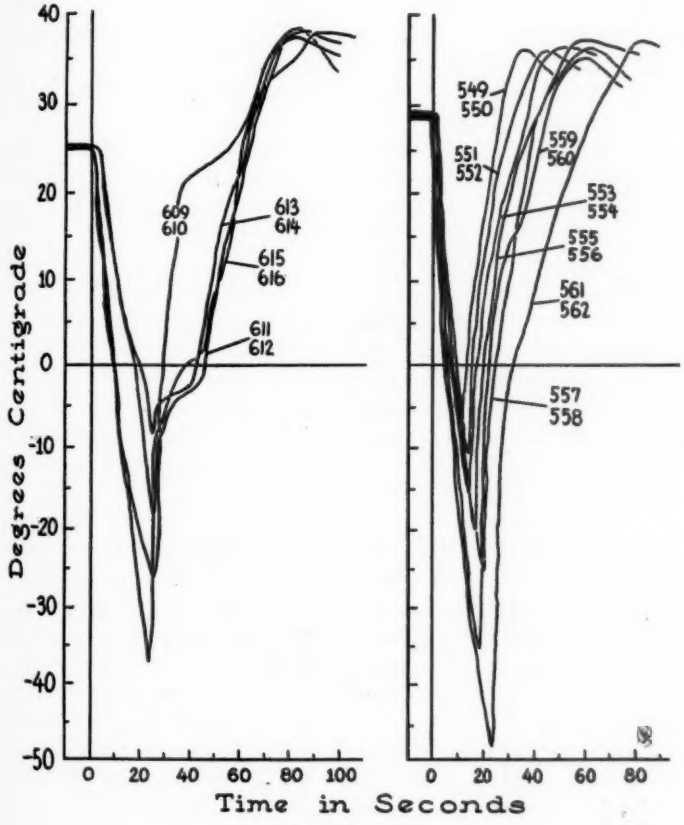


FIGURE III

simultaneous freezing and thawing in Gey's balanced salt solution versus ascitic fluid. The potentiometric record is shown in figure III while the results are summarized in table 3. It will be seen that the effect of the freezing medium again did not produce clear evidence for any differential effect in cell survival.

Out of a total of 40 experiments (Fig. IV), outgrowth of spindle cells appeared to be markedly enhanced in 7 tests in which freezing was achieved in Gey's solution while in 7 other instances this medium appeared to favor the outgrowth of various cells. No striking difference between results was obtained as a consequence of the use of the two media in 25 experiments while ascitic fluid appeared to offer some slight differential beneficial action in one experiment. It should be noted that the less damaging slower freezing may account for the better survival from explants used in experiments 111 and 112.

Given this variability it would appear that within the limits of error inherent in the method employed, the difference in results between freezing in Gey's balanced salt solution and in a protein containing medium is not clearly significant but is slightly in favor of the Gey's solution.

It should be noted that in the evaluation of results obtained with tissue culture methods it is well known that whenever epithelium or muscle fibers are discouraged, spindle cells emigrate more profusely and that the converse relationship is also true. This factor of cellular competition which is probably related both to the physical effects of population crowding and to the availability of nutrients becomes important in influencing the direction of cellular outgrowth.

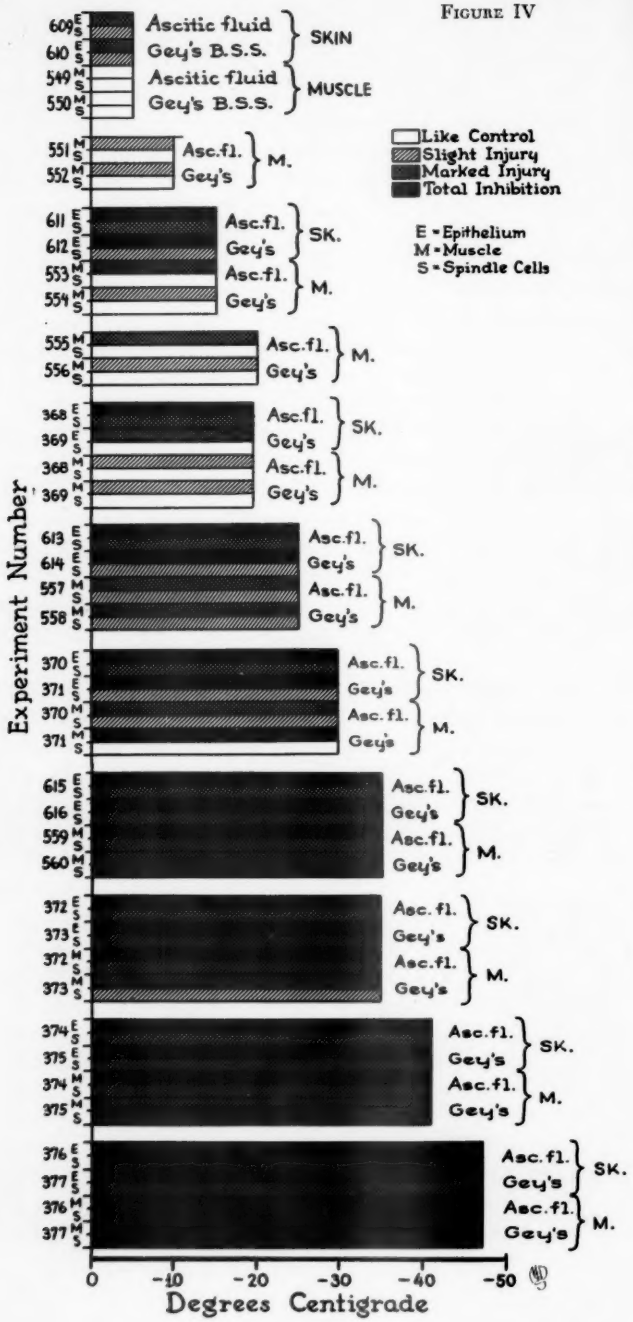
In these as well as other experiments in this series, the relatively high degree of variability of results suggests that more reproducible data might be obtained with pure cell strains. Moreover, primary explants have the great disadvantage that a considerable percentage of cells are dead or dying as a consequence of cutting operations. On the other hand, testing cellular injury with the use of primary explants has the advantage that the stress is imposed upon parenchymatous elements in relation to corresponding stromal cells and that both lie imbedded in their native ground substance. In the hands of Gey and his associates (1954), important reciprocal dependencies have been found to exist between lymphosarcomatous cells and corresponding fibroblasts.

TABLE 3

Results Obtained From 12-day Chick Embryo Explants

Expt. No.	FROZEN IN ASCITIC FLUID Skin		Leg Muscle		Expt. No.	FROZEN IN GEY'S BALANCED SALT SOLUTION Skin		Leg Muscle	
CONTROL—	Epithelium	18/18	Muscle	18/20	CONTROL—	Epithelium	9/20	Muscle	19/20
unfrozen	Spindles	18/18	Spindles	20/20	unfrozen	Spindles	20/20	Spindles	20/20
Expt. 609	Epithelium	1/20			Expt. 610	Epithelium	1/18		
(skin)	Spindles	9/20			(skin)	Spindles	14/18		
Expt. 549			Muscle	16/20		No outgrowth	6/18		
(muscle)			Spindles	20/20	Expt. 550			Muscle	18/20
–5.0° C.					(muscle)			Spindles	20/20
					–5.0° C.				
Expt. 551			Muscle	11/20	Expt. 552			Muscle	14/20
–10.0° C.			Spindles	20/20	–10.0° C.			Spindles	20/20
Expt. 611	Epithelium	0/20			Expt. 612	Epithelium	0/20		
(skin)	Spindles	7/20			(skin)	Spindles	13/20		
	No outgrowth	13/20				No outgrowth	7/20		
Expt. 553			Muscle	7/19	Expt. 554			Muscle	16/20
(muscle)			Spindles	17/19	(muscle)			Spindles	20/20
–15.0° C.			No outgrowth	2/19	–15.0° C.				
Expt. 555			Muscle	5/20	Expt. 556			Muscle	8/20
–20.0° C.			Spindles	19/20	–20.0° C.			Spindles	18/20
			No outgrowth	1/20				No outgrowth	2/20
Expt. 613	Epithelium	0/19			Expt. 614	Epithelium	0/20		
(skin)	Spindles	6/19			(skin)	Spindles	13/20		
	No outgrowth	13/19				No outgrowth	7/20		
Expt. 557			Muscle	5/20	Expt. 558			Muscle	7/20
(muscle)			Spindles	15/20	(muscle)			Spindles	13/20
–25.0° C.			No outgrowth	5/20	–25.0° C.			No outgrowth	7/20
Expt. 615	Epithelium	0/19			Expt. 616	Epithelium	0/20		
(skin)	Spindles	1/19			(skin)	Spindles	2/20		
	No outgrowth	18/19				No outgrowth	18/20		
Expt. 559			Muscle	0/20	Expt. 560			Muscle	4/20
(muscle)			Spindles	9/20	(muscle)			Spindles	14/20
–35.0° C.			No outgrowth	11/20	–35.0° C.			No outgrowth	6/20
Expt. 561			No outgrowth	20/20	Expt. 562			Spindles	1/20
–49.0° C.					–49.0° C.			No outgrowth	19/20

FIGURE IV


609 E S
610 E S
549 M S
550 M S
Ascitic fluid
Gey's B.S.S.
Ascitic fluid
Gey's B.S.S.
SKIN
MUSCLE
551 M S
552 M S
Asc.fl.
Gey's
M.
Like Control
Slight Injury
Marked Injury
Total Inhibition
E = Epithelium
M = Muscle
S = Spindle Cells
611 E S
612 E S
553 M S
554 M S
Asc.fl.
Gey's
Asc.fl.
Gey's
SK.
M.
555 M S
556 M S
Asc.fl.
Gey's
M.
368 E S
369 E S
368 M S
369 M S
Asc.fl.
Gey's
Asc.fl.
Gey's
SK.
M.
613 E S
614 E S
557 M S
558 M S
Asc.fl.
Gey's
Asc.fl.
Gey's
SK.
M.
370 E S
371 E S
370 M S
371 M S
Asc.fl.
Gey's
Asc. fl.
Gey's
SK.
M.
615 E S
616 E S
559 M S
560 M S
Asc.fl.
Gey's
Asc.fl.
Gey's
SK.
M.
372 E S
373 E S
372 M S
373 M S
Asc. fl.
Gey's
Asc.fl.
Gey's
SK.
M.
374 E S
375 E S
374 M S
375 M S
Asc.fl.
Gey's
Asc.fl.
Gey's
SK.
M.
376 E S
377 E S
376 M S
377 M S
Asc.fl.
Gey's
Asc.fl.
Gey's
SK.
M.
Experiment Number
0
-10
-20
-30
-40
-50
Degrees Centigrade

According to Fell (personal communication), the reaction of organ cultures to hormones and vitamins appears to reflect more nearly the reactions of whole animals than do emigrating cells in disorganized sheets. It follows, therefore, that the effect of cold injury may be studied with advantage on living systems at various levels of organization. As an experimental instrument, the primary explant is useful at a point somewhere between what obtains in the organ as a whole and what can be learned from the analysis of series of related pure cell strains.

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THE EFFECT OF VIRUSES ON HUMAN NEOPLASMS IN TISSUE CULTURES*

MORRIS POLLARD, ROBERT BUSSELL, AND CLIFFORD C. SNYDER

Some of the neurotropic viral agents exert an oncolytic effect on experimental tumors of rodents (Levaditi, *et al.*, 1937; Haber and Coquoin 1937; Moore 1949, 1951; Buckley, *et al.*, 1951; Koprowski and Koprowska 1952; Koprowski and Love 1953) and of chickens (Sharpless, *et al.*, 1950). By tissue culture studies, a susceptibility spectrum of rodent tumors to various neurotropic viral agents has been demonstrated (Pollard and Bussell, 1952) in which certain of them exert either a marked cytolytic effect, little demonstrable effect, or what appears to be a growth promoting effect. In this respect, it was felt that the reaction of human tumors to virus infection should be similarly studied. Human cases of neoplastic disease have been treated with viral agents, but marked beneficial effect has not yet been noted (Southam and Moore, 1951, 1952; Koprowska, 1953). Most of the viruses so used were relatively inocuous to the host and, except for Bunyamwera virus, barely induced clinical signs of infection.

The tissue culture studies reported here, with surgically excised human tumors demonstrate a spectrum of viral susceptibility which is similar to that observed by us with tumors from rodents.

Methods and Materials

Surgically excised human malignant tumors were explanted into roller tube cultures, as previously described (Pollard and Bussell, 1952). The nutrient fluid contained either ox serum ultrafiltrate (UF*) or human ascitic fluid (AF). When each tumor explant demonstrated consistent evidence of outgrowth, the nutrient fluid was exchanged for UF medium and virus. The dosage of virus (0.05 ml.) used for each tube contained approximately 1000 LD_{50} or 100 hemagglutinin units of virus. At least four tubes, each with 4 tumor explants, were studied with each virus. The subsequent growth pattern of each explant was followed by daily examination at low magnification. Nutrient fluids were changed at 4 day intervals, and otherwise the pH of the fluids were adjusted when necessary.

The human tumors studied by the inoculation of viruses into explants thereof are listed on table 1. The neurotropic viruses used in these studies

* From the Virus Research Laboratory, The University of Texas, Medical Branch, Galveston. Received for publication January 15, 1954.

were propagated in mouse brain with the exception of Lansing poliomyelitis virus (LCR) which was prepared from infected cotton rat brain tissues. The virus strains are identified as follows: St. Louis encephalitis (Hubbard), Neuroptropic influenza A (WS), yellow fever (17D), rabies (CVS), and encephalomyocarditis (EMC). Newcastle disease virus (California) was propagated in the fluids of developing chick embryos.

Results

St. Louis encephalitis virus (SLE) destroyed the explants of 7 of the 10 tumors studied (Table 1). It failed to affect an ovarian tumor, a melanoblastoma, and one squamous cell carcinoma. Five other tumors failed to grow well enough to warrant inoculation with the virus. The oncolytic effect of SLE virus appeared on the first and second days after inoculation and most of the infected explants appeared dead by the second to fifth days. The cytolytic effect appeared at the periphery of each explant and progressed centrally until all of the tissue appeared dead. SLE induced oncolysis occurred in such diverse tumor types as parotid tumor, adenocarcinoma, and squamous cell carcinoma.

LCR virus caused extensive degenerative changes in tumor explants on the second and third days following inoculation; however, healthy fibrocytes were observed around each explant during the entire period of observation, which might be interpreted as incomplete oncolysis (Table 1). Neurotropic influenza A virus

TABLE 1

Tumor	Source	Initial growth	Virus inoculated and results				
			SLE	Neuro-Inf.	LCR	YF	Rabies
Fibrosarcoma	Primary	NG*					
Melanoblastoma	Primary	NG					
Melanoblastoma	Node	G	2/	2/		GG	
Parotid tumor	Node	G	2/7†	2/			GG
Adeno CA	Ascitic fluid	G	1/2				GG
Adeno CA	Ascitic fluid	G	2/4	3/	2/	GG	
Squamous cell CA	Node	NG					
Squamous cell CA	Node	G	2/4		3/		
Squamous cell CA	Primary	NG					
Squamous cell CA (Anaplastic)	Primary	G	1/4			GG	
Squamous cell CA	Primary	G	PG	G	PG		
Hodgkins	Ascitic fluid	G	1/3	G			
Myxoma	Primary	NG					
Sarcoma	Node	G	2/5	3/	3/	GG	
Leucosarcoma	Node	G		3/	2/		
Ovarian tumor	Primary	G	G	G			

*NG—no growth, SG—some growth, G—growth, GG—enhanced growth.
†Day degeneration appeared/day explants dead.

caused some degenerative changes in tumor explants, but the oncolytic process was slower and was incomplete. Its end effect was similar to that noted above with LCR virus. EMC virus induced some inconclusive degenerative changes in two of four tumor specimens. Newcastle disease virus induced no change in growth patterns.

Rabies and yellow fever viruses appeared to stimulate the growth rate of all the tumor explants to which they were added. Growing tumor tissue caused the nutrient fluid to turn acid and the interval for such a change to occur reflected the relative activity of the tissue explants. With viral oncolysis, this fluid remained alkaline; whereas with enhanced growth the fluid was acid within 24 hours.

The fate of each virus in parasitized tumor tissue was not determined.

Discussion. While SLE virus destroyed many of the human tumor explants *in vitro*, it provides no rationale for anticipating its effect *in vivo*. The cytolytic response to SLE infection in the intact host may be influenced by accessibility of virus to tumor tissue and by the immunological response of the host to such infection. Previous studies by us have indicated that SLE virus disappears from explanted lymphatic tissue on the 4th and 5th days following inoculation, and that it can be demonstrated in growing fibrocytes for over one week.

The observations of Southam and Moore (1951, 1952) would indicate that ultimate oncolytic benefit was not derived from *in vivo* infections with Ilheus, West Nile, Egypt 101 and Bunyamwera viruses. The tumors were not examined for virus susceptibility prior to inoculation, and of course, such examination might actually be of little benefit. Koprowski (1953) studied the cytolytic effect of West Nile virus on human peritoneal and pleural tumors by examining aspirated tumor cells at regular intervals after inoculation. While a persistent viremia was detected in 4 of 5 patients and while some tumors appeared altered, such treatment was of little benefit to the patients. Pollard and Snyder (1954) observed the *in vivo* effect of neurotropic influenza A and of Western equine encephalomyelitis viruses on a case of squamous cell carcinoma. While a viremia with the latter virus was noted, little objective advantage to the patient could be noted.

Since the human tumors reported on herein were not uniformly susceptible even to an agent as virulent as SLE, one should avoid arbitrary selection of a possible therapeutic viral agent. The growth enhancing effect of rabies and of yellow fever viruses on tumors *in vitro*, aside from the severity of their infections, might discourage their consideration as oncolytic agents for humans. SLE virus quickly destroyed such diverse tumor types as adenocarcinomas, a parotid tumor, and squamous cell carcinomas. In the latter, the tumor cells were destroyed while the fibrocytes of the matrix continued growing, thus indicating some degree of selectivity on the tissue being parasitized. The fibrocytes that survived LCR and influenza infection may also indicate some evidence of tumor selectivity by the infecting virus. Thus far it appears that viruses, destructive to experimental tumors, exert little benefit to the human patients that have thus far been inoculated.

The observations reported on here were more qualitative than quantitative; therefore, only complete destruction of a tumor explant was acceptable as evidence of oncolysis. On this basis, SLE virus was the only effective agent of those here studied.

Summary. SLE virus caused complete destruction of tissue culture explants of 6 of 10 human tumors. The oncolytic effect on such tumors of Lansing poliomyelitis virus and of neurotropic influenza A virus was incomplete. Rabies and yellow fever viruses seemed to stimulate the growth of tumor explants, The tissue culture technique described herein might provide a means of pre-determining the susceptibility of tumors to viral agents, thus guiding their possible application as therapeutic agents.

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TREATMENT OF A HUMAN CANCER CASE WITH VIRUSES

MORRIS POLLARD AND CLIFFORD C. SNYDER*

In the course of studying viral oncolysis, we inoculated two different types of viruses into a human case of squamous cell carcinoma, and thereafter traced the cytological and serological responses of the patient to these inoculations.

E. B., age 40, white male, developed a tumorous growth on the hard palate which he neglected for 12 months. When finally examined, his condition was diagnosed as squamous cell carcinoma, Grade III (Fig. I).[1] Extensive surgical resection of involved tissues plus extensive X-irradiation therapy failed to arrest the progress of the neoplasm. When it was obvious to both the patient and to his attending physicians that the prognosis was hopeless, both parties requested that viral therapy be considered.

Biopsy specimens of primary tumor tissue and of lymph nodes with metastases were explanted to roller tube tissue cultures. The neoplasm grew out as distinct epithelial cells. Sets of 6 tubes each containing four growing explants were inoculated with one of the following viruses: Neurotropic influenza A (WS), Western equine encephalomyelitis (Cal.), St. Louis encephalitis (Hubbard), rabies (CVS), and yellow fever (17 D). The technique has been described by Pollard and Bussell (1952). Western equine (WEE) and St. Louis encephalitis (SLE) viruses destroyed the tumor explants on the second and third days; and influenza virus caused degenerative changes on the fourth day. Rabies and yellow fever viruses appeared to induce enhancement of tumor growth.

On the basis of the viral susceptibility spectrum demonstrated above, influenza virus was selected as the first inoculum. This choice was motivated primarily by the view that influenza virus is not as lethal for humans as are WEE and SLE viruses. Influenza virus was inoculated directly into the tumor at multiple sites. The inoculum contained 100,000 mouse I.C. LD_{50} of virus. Subsequent observations of the patient over a period of 8 days failed to demonstrate any beneficial effect. The patient was then inoculated in similar manner with two million mouse LD_{50} of WEE. At no time following the virus inoculations did the patient manifest any symptoms suggestive of an infection either of the

* From The University of Texas, Medical Branch, Galveston. Submitted for publication February 25, 1954.

[1] Biopsy specimen examined by Dr. Richard McDonald, The University of Texas, Medical Branch Hospitals.

4 days after WEE inoculation

Prior to virus inoculation

FIG. 1. Squamous cell carcinoma X 280.

central nervous system or otherwise. In fact, the patient claimed that he felt considerably improved but this was attributed more to a psychological boost than to any apparent physical improvement. The physical condition of the patient was not such as would suggest an encouraging prognosis at any time during the course of this study.

Citrated blood specimens were collected from the patient on every day following the inoculation of WEE and each was inoculated intracerebrally into mice. A WEE viremia was demonstrable for the first 2 days following the inoculation, and therefore the blood was not infective (Chart I). The viremia level was not titrated.

Four days following the WEE inoculation the superficial tumors appeared and felt softened. A biopsy specimen, collected at this time, was negative for WEE virus and was described histologically as a poorly differentiated squamous cell carcinoma.[2] The photomicrograph (Fig. I) appears rather indistinct and cellular margins are poorly defined; however, this was typical of the specimen examined. Serum specimens, collected at weekly intervals following the inoculations, demonstrated a rise in hemagglutinin—inhibition titer for influenza A. A similar test for influenza B was negative. Complement fixation tests for WEE revealed a rise in titer 9 days subsequent to inoculation of the virus (Chart I).

CHART I. *Response of Human Cancer Patient to Virus Inoculation.*

Inoculation:	Inf. A		WEE									
Days	0	1 to 7	8	9	10	11	12	13	14	15	16	17
HI* titer												
Inf. A	1:40	...	1:80	...	...	...	...	...	...	...	...	1:320
Inf. B	Neg.	...	Neg.	...	...	...	...	...	...	...	...	Neg.
CF titer												
WEE	Neg.	...	...	...	...	...	...	...	...	...	...	1:40
Viremia												
WEE	...	...	...	5/5†	4/4	0/6	0/8	0/8	0/4	...	...	...

*Hemagglutination inhibition.
†Number of mice infected/number inoculated.

One week following the inoculation of WEE virus, the neoplasm resumed its growth and the patient expired one week later. Permission for autopsy was refused.

[2] Examined by Dr. John H. Childers, The University of Texas, Medical Branch Hospitals.

4 days after WEE inoculation

FIG. I. Squamous cell carcinoma X 280. Prior to virus inoculation

Viral oncolysis in the human has been studied rather intensively (Southam and Moore, 1951; 1952; Koprowska, 1953; Newman and Southam, 1954); however, thus far the course of illness has not been influenced beneficially. The tumor studied here was susceptible to the viral oncolytic process when inoculated as explants in tissue culture, but susceptibility to 2 of the same viruses was not duplicated *in vivo*. In view of the WEE viremia manifested, we may assume that this virus did propagate in the host, and, as expected, an immunological response was detected 7 days later. The WEE agent used here had been passaged through numerous mice and may have lost its avidity for the intact human organism. It killed mice and 9-day chick embryos regularly within 48 hours after inoculation.

Chart I indicates that the patient already possessed specific antibody to influenza A virus when he was inoculated. If an oncolytic effect could have been exerted by this virus, its effect might have been mitigated by the immunity already present in the host. It is obvious that infections with neurotropic influenza A and with WEE viruses failed to influence the course of the neoplastic disease in the one case reported on herein.

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THERMAL STRESS AND TISSUE CULTURE
IV. The Intensity-Time Factor*

C. M. Pomerat, J. Nakai,† and P. F. Gilliland

It was pointed out by Lewis (1951) that long exposure to temperatures only a few degrees less than that of the body may produce tissue pathology identical with what is observed in true frostbite. Preliminary experiments on the relation of freezing intensity to length of exposure were reported by Pomerat and Lewis (1954). Considerably greater injury was observed to result from freezing for 30 minutes as compared to 5 minutes at comparable temperatures. In order to obtain more data with tissue culture techniques regarding these relationships experiments were designed to provide comparable treatment for parallel test series where the sole variable consisted of tissue storage in a deep freeze unit versus storage in an ordinary ice box. Test tubes containing 20 explants of each tissue species under study were removed at suitable intervals for culture in order to determine the minimal time required to produce graded injury and total inhibition for each of the storage temperatures. The essential results obtained are represented in table 1 and in figures I and II.

In the first group of experiments freezing of 12-day chick tissues indicated that skin might be more vulnerable than muscle under conditions of storage in the deep freezer at —16.0° C. Epithelium was damaged following as little as 1½ hours and totally inhibited after 3 hours. This difference in time appeared to be sufficient to change the spindle cell outgrowth from a reading of "like control" to "total inhibition." Both muscle fiber and spindle cell outgrowth showed no injury with as much as 3 hours' exposure but after 6¾ hours the fibers were badly injured and the spindle elements were slightly retarded. Total inhibition of muscle outgrowth resulted after 20 hours' exposure while this effect on spindle cells occurred between 20 and 72 hours.

* From the Tissue Culture Laboratory, Medical Branch, The University of Texas. This study was supported by funds provided under Contract No. AF 18(600)–559 with the USAF School of Aviation Medicine, Randolph Field, Texas.

† Japanese Ministry of Education fellow, from the Department of Anatomy, University of Tokyo Medical School.

TABLE 1

Protocols For 3 Series of Experiments Involving the Intensity-Time Factor For Freezing 12-Day Chick Embryo Tissues

Expt. No.	SERIES I	SKIN				LEG MUSCLE			
CONTROL—unfrozen		Epithelium 13/17 Spindles 17/17				Muscle 19/20 Spindles 20/20			
		—16.0° C.		+ 7.0° C.		—16.0° C.		+ 7.0° C.	
Expt. 192 1½ hrs.	.....	Epithelium Spindles	2/20* 20/20	Epithelium Spindles	6/10 10/10	Muscle Spindles	20/22 22/22	Muscle Spindles	16/19 9/19
Expt. 193 3 hrs.	.....	No outgrowth	20/20	Epithelium Spindles	15/20 20/20	Muscle Spindles	20/20 20/20	Muscle Spindles	15/20 20/20
Expt. 194 6¾ hrs.	.....	No outgrowth	20/20	Epithelium Spindles	12/20 20/20	Muscle Spindles No outgrowth	7/19 16/19 3/19	Muscle Spindles	20/20 20/20
Expt. 195 20 hrs.	.....	No outgrowth	20/20	Epithelium Spindles	7/20 20/20	Muscle Spindles No outgrowth	0/20 1/20 19/20	Muscle Spindles	19/20 20/20
Expt. 196 72 hrs.	.....	No outgrowth	20/20	Epithelium Spindles	1/20 20/20	No outgrowth	20/20	Muscle Spindles No outgrowth	10/19 17/19 2/19
Expt. 197 144 hrs.	.....	No outgrowth	20/20	Epithelium Spindles	0/20 17/17	No outgrowth	20/20	Muscle Spindles	0/15 15/15
Expt. 198 288 hrs.	.....	No outgrowth	20/20	No outgrowth	20/20	No outgrowth	20/20	No outgrowth	20/20

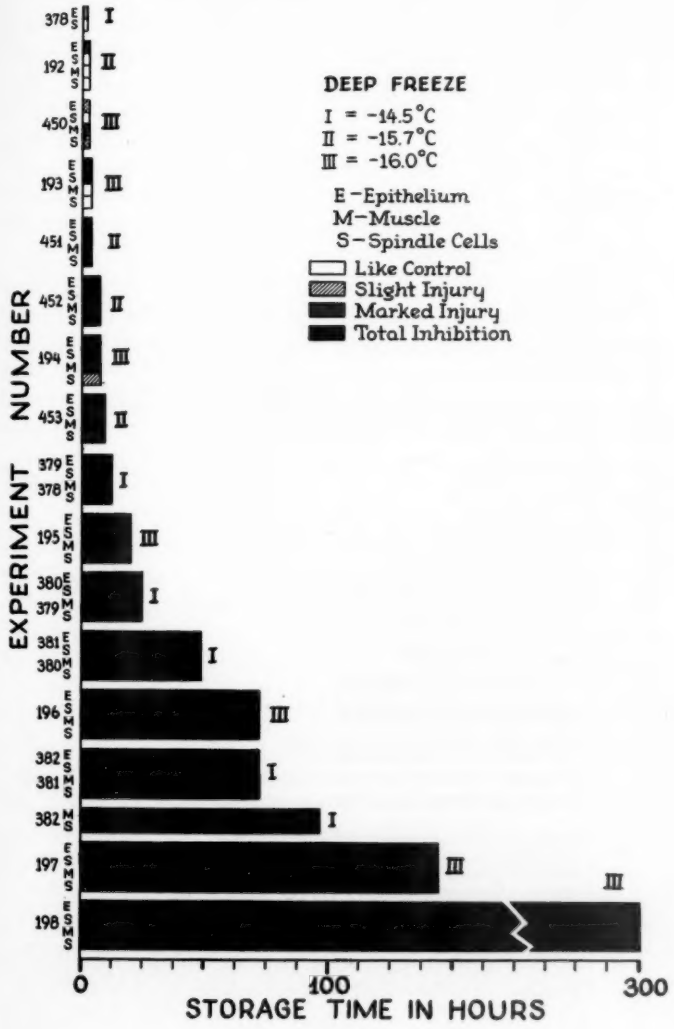
Expt. No.	SERIES II		SKIN				LEG MUSCLE			
CONTROL—unfrozen			Epithelium Spindles	19/20 20/20			Muscle Spindles	19/20 20/20		
			—14.5° C.		+ 4.0° C.		—14.5° C.		+ 4.0° C.	
Expt. 378										
—14.5° C.	Skin	1 hr.	Epithelium	8/16	Epithelium	11/20	Muscle	12/20	No outgrowth	20/20
	Muscle	12 hrs.	Spindles	16/16	Spindles	20/20	Spindles	19/20		
+ 4.0° C.	Skin	48 hrs.					No outgrowth	1/20		
	Muscle	72 hrs.								
Expt. 379										
—14.5° C.	Skin	12 hrs.	Epithelium	1/20	No outgrowth	20/20	No outgrowth	20/20	No outgrowth	20/20
	Muscle	24 hrs.	Spindles	19/20						
+ 4.0° C.	Skin	72 hrs.	No outgrowth	1/20						
	Muscle	96 hrs.								
Expt. 380										
—14.5° C.	Skin	12 hrs.	Epithelium	0/20	No outgrowth	20/20	No outgrowth	20/20	No outgrowth	20/20
	Muscle	24 hrs.	Spindles	19/20						
+ 4.0° C.	Skin	72 hrs.	No outgrowth	1/20						
	Muscle	120 hrs.								
Expt. 381										
—14.5° C.	Skin	48 hrs.	Epithelium	0/20	No outgrowth	20/20	No outgrowth	20/20	No outgrowth	20/20
	Muscle	72 hrs.	Spindles	9/20						
+ 4.0° C.	Skin	144 hrs.	No outgrowth	11/20						
	Muscle	144 hrs.								
Expt. 382										
—14.5° C.	Skin	72 hrs.	No outgrowth	20/20	No outgrowth	20/20	No outgrowth	20/20	No outgrowth	20/20
	Muscle	96 hrs.								
+ 4.0° C.	Skin	168 hrs.								
	Muscle	168 hrs.								

TABLE 1—(Continued)

Protocols For 3 Series of Experiments Involving the Intensity-Time Factor For Freezing 12-Day Chick Embryo Tissues

Expt. No.	SERIES III	SKIN				LEG MUSCLE			
CONTROL— unfrozen		Epithelium Spindles	14/24 24/24			Muscle Spindles	17/17 17/17		
		−15.7° C.		+ 2.0° C.		−15.7° C.		+ 2.0° C.	
Expt. 444 + 2.0° C.	6 hrs.			Epithelium Spindles	12/18 18/18			Muscle Spindles	15/20 20/20
Expt. 450 −15.7° C.	1½ hrs.	Epithelium Spindles	6/10 10/10	Epithelium Spindles	8/12 12/12	Muscle Spindles	1/10 6/10	Muscle Spindles	19/22 22/22
Expt. 445 + 2.0° C.	9 hrs.					No outgrowth	4/10		
Expt. 451 −15.7° C.	3 hrs.	No outgrowth	12/12	Epithelium Spindles	2/16 16/16	No outgrowth	15/15	Muscle Spindles	2/20 20/20
Expt. 446 + 2.0° C.	24 hrs.								
Expt. 452 −15.7° C.	6 hrs.	No outgrowth	17/17	Epithelium Spindles	9/10 10/10	No outgrowth	20/20	Muscle Spindles	14/15 15/15
Expt. 447 + 2.0° C.	36 hrs.								
Expt. 453 −15.7° C.	9 hrs.	Epithelium Spindles	0/7 3/7	Epithelium Spindles	8/14 13/14	Muscle Spindles	0/20 2/20	Muscle Spindles	14/14 14/14
Expt. 448 + 2.0° C.	48 hrs.	No outgrowth	4/7	No outgrowth	1/14	No outgrowth	18/20		

*Out of 20 explants 2 showed epithelial outgrowth.


DEEP FREEZE
I = -14.5°C
II = -15.7°C
III = -16.0°C
E – Epithelium
M – Muscle
S – Spindle Cells
Like Control
Slight Injury
Marked Injury
Total Inhibition
EXPERIMENT NUMBER
378 E S I
192 E M S II
450 E M S III
193 E M S III
451 E M S II
452 E M S II
194 E M S III
453 E M S II
379 E M S
378 E M S I
195 E M S III
380 E M S
379 E M S I
381 E M S
380 E M S I
196 E M S III
382 E M S
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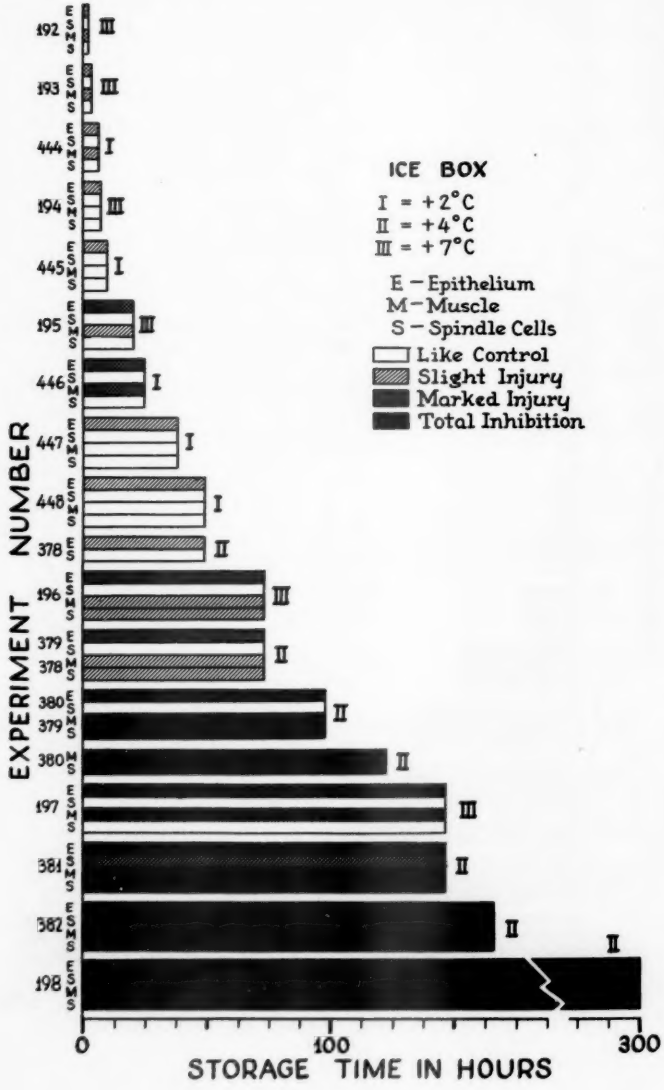
198 E M S III
0
100
300
STORAGE TIME IN HOURS

FIGURE I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192 E S M S III
193 E S M S III
444 E S M S I
194 E S M S III
445 E S M S I
195 E S M S III
446 E S M S I
447 E S M S I
448 E S M S I
378 E S II
196 E S M S III
379 E S M S II
378 M S
380 E S M S II
379 M S
380 M S II
197 E S M S III
381 E S M S II
382 E S M S II
198 E S M S II
ICE BOX
I = +2°C
II = +4°C
III = +7°C
E – Epithelium
M – Muscle
S – Spindle Cells
Like Control
Slight Injury
Marked Injury
Total Inhibition
0
100
300
STORAGE TIME IN HOURS

FIGURE II

In contrast, corresponding tissues stored in an ice box at +7.0° C. showed slight injury to epithelium after 1½, 3, and 6¾ hours while after 20 and 72 hours marked inhibition of outgrowth was noted. Total inhibition of epithelial cell migration occurred between 72 and 144 hours. Slight injury was obtained for muscle fibers in from 1½ to 72 hours with total inhibition being observed after 144 hours. No injury to spindle cells from muscle explants was found after 144 hours but total inhibition resulted within 288 hours of storage in the ice box.

By way of adding data to this form of experiment with the use of slightly different temperatures, a second series of observations was made also using 12-day chick skin and muscle. At —14.5° C. in the deep freezer, epithelium was slightly injured after one hour and totally inhibited following 12 hours of storage. Spindle cells from skin resembled those of the controls after one hour but were totally inhibited after 12 hours' exposure. Both fibers and spindle cells from muscle explants were totally inhibited after 12 hours.

In contrast, corresponding tissues in an ice box at +4.0° C. showed considerably less injury. Epithelium from skin showed only slight injury after 48 hours, marked damage following 72 hours, and total inhibition by 96 hours. Corresponding spindle cells showed otugrowth like the control following 96 hours' exposure, marked injury after 144 hours, and total inhibition by 168 hours.

In a third series tissues were stored at both —15.7° C. and +2.0° C. At —15.7° C. epithelium was slightly injured after 1½ hours and totally inhibited by 3 hours. Corresponding spindle cells from skin explants showed no injury at 1½ hours. While total inhibition was found for the 3 and 6 hour periods, some spindle cells survived 9 hours of storage.

Emigration of muscle fibers was markedly retarded by storage at —15.7° C. for 1½ hours and totally inhibited following 3 hours. Spindle cells from these muscle explants appeared to be slightly more resistant to 1½ hours' storage but were also totally suppressed by 3 hours.

Under conditions of storage at +2.0° C. in an ice box, epidermal cells were only slightly retarded after 48 hours. However, for some unexplained reason the set tested for viability after 24 hours

showed considerably more injury. Muscle explants did not regularly show damage following storage at +2.0° C. for two days with the exception of some degree of retardation in the muscle fiber emigration of the series at 6 and 24 hours.

The results from the three experiments stored in the deep freezer have been collected in figure I and the corresponding set exposed to ice box temperature in figure II.

Epidermal cells did not survive beyond 3 hours at deep freeze temperatures between —14.5 to —16.0° C., while at ice box temperatures of from +2.0 to +7.0° C. they ceased to show emigration between 72 and 96 hours. Muscle fibers under similar temperature exposures gave somewhat variable results but the survival limit appeared to lie between 6¾ hours and 9 hours in the deep freezer, while in the ice box the thermal death point was between 72 and 96 hours.

The critical time for inhibition of spindle cells of dermal origin lay at 9 and 12 hours in the deep freezer as compared to 144 to 168 hours in the ice box. In contrast, spindle cells from the muscle explants were irreversibly damaged between 20 and 24 hours while in the ice box total injury occurred between 144 and 168 hours.

Despite a considerable degree of variation, these results serve to prove the importance of the intensity-time factor in freezing and thawing.

Summary

The relation between intensity and time of freeing is given in the table showing the time in hours required to produce total inhibition of cellular emigration following storage at two different levels of temperature.

	Deep freezer —14.5° C. to —16.0° C.	Ice box +2.0° C. to +7.0° C.
Skin		
Epidermal cells	1½– 3 hrs.	72– 96 hrs.
Spindle cells	9 –12 hrs.	144–168 hrs.
Muscle		
Muscle cells	6¾– 9 hrs.	72– 96 hrs.
Epidermal cells	20 –24 hrs.	144–168 hrs.

Under the conditions of these experiments injury was greater both with increase of time of storage as well as cold intensity.

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THERMAL STRESS AND TISSUE CULTURE

V. Efforts to Enhance Outgrowth Following Cold Injury With the Use of Rimifon, Marsilid and Various Cortisone Preparations*

C. M. Pomerat, J. Nakai,† and T. G. Blocker, Jr.

There is a healthy skepticism in many surgical circles that no chemical compound can be employed topically or systemically which will enhance the reparative capacity of cells beyond that which can be achieved naturally under ideal physiological conditions in an individual whose nutritional state is satisfactory. The tissue culture method is ideally suited for explorations on the growth stimulating propensity of substances which may be introduced into an isolated system in which the variables can be controlled with a reasonable degree of precision. In view of the importance of finding specific tissue growth-promoting materials, it is believed that considerable effort should be invested in this quest. Following upon studies by Allgöwer, Pomerat, and Blocker (1952), this laboratory has continued to screen all drugs which appear to be reasonably promising as aids to epithelialization. The methods employed and the findings obtained should be published at least in brief form, even though they be negative in character.

The techniques which have been developed to obtain reproducible thermal injuries to tissue fragments as measured by their capacity to exhibit various degrees of cellular outgrowth in tissue culture appear to offer a valuable tool in the quest for new therapeutic agents. Test drugs at various concentrations are added directly in the fluid nutrient in parallel with suitable untreated controls. All individual data entries reported are based on 20 explants maintained for 6 days *in vitro*.

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Rimifon and Marsilid[1]

These are members of a series of antituberculous drugs developed by the Roche Research Laboratories which have been under clinical trial since June, 1951. Chemically, Rimifon is isonicotinylhydrazine, while Marsilid is 1-isonicotinyl-2-isopropylhydrazine. These are obtained as white crystals which are very soluble in water. The dry powder is stable and sterilization of the aqueous solution can be effected by autoclaving for 20 minutes at 15 pounds pressure at pH 6 to pH 8. Both are hydrazides of isonicotinic acid. The LD_{50} by oral administration for the rat had been found to be 1485 mg./kg. for Rimifon and 383 mg./kg. for Marsilid. In chronic administration both compounds produced lowering of the hemoglobin concentration but both appeared to offer promise as antituberculous agents.

Following a preliminary report, Bosworth, Wright, and Fielding (1952) found additional evidence for the value of Marsilid in the management of bones and joint tuberculosis, especially in patients who had been receiving Rimifon. A dosage of 4 milligrams per kilo was suggested with the recommendation that this be reduced to 2 mg./kg. if clonus developed. Unfortunately, central and sympathetic neurotoxic symptoms were frequently encountered. The clinical impression that tuberculous sinuses healed more rapidly under this treatment aside from the bacteriostatic effect led these workers to postulate that Marsilid might prove valuable for the stimulation of mesodermal tissues generally. The increase of capillary proliferation and of granulation response suggested that the effect of these hydrazides of isonicotinic acid should be studied with tissue culture methods.

Toxicity to Cells in Tissue Culture. As a preliminary step in the study of the action of chemicals on cells in tissue culture, it has been the standard practice in this laboratory to determine the minimal concentration required to produce total inhibition (MID) and the amount necessary to show slight injury as compared to untreated control cultures (LID). The methods employed, together with a table of values for 110 chemicals, have been recently described by Pomerat and Leake (1954).

The data obtained for the MID and LID of both Rimifon and Marsilid with the use of approximately 500 explants of three species of chick tissues are summarized in figure I.

[1] Kindly supplied by Hoffmann-La Roche Inc., Nutley, N. J.

The Effect of Rimifon and Marsilid on Cultures of Human Rib Marrow.[2] Using marrow obtained from ribs removed in the course of thoracic surgery, Woodard and Pomerat (1953) found that in a relatively high percentage of cultures patent vessels could be obtained within about one week of incubation. This test object appeared to be unusually well suited for an examination of the capacity of drugs to stimulate capillary proliferation.

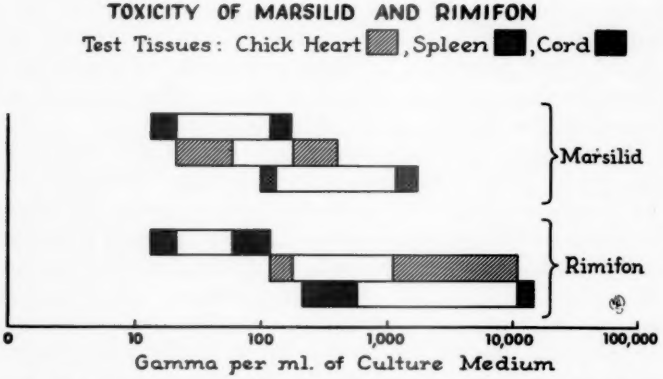

FIG. I. The left-hand block in each bar is used to represent the LID (least injurious dose) while the right-hand block refers to the MID (maximal or dose for total injury). Outgrowth like that of untreated control cultures was obtained with the value represented by the left edge while cell migration was slightly inhibited by the amount indicated by the right edge of the LID block. Almost total inhibition of outgrowth resulted from the dosage represented by the left-hand edge and no outgrowth resulted in culture containing the amount shown by the right margin of the MID block.

Thirty roller tubes were set up in the usual manner, but in 10 tubes a control fluid phase was used while in 10 others 25 γ/ml. Marsilid and in 10 more 50 γml. Rimifon were added.

After only three days of incubation, one tube of each group was stained. At that time some vessels were already visible in the control and Marsilid preparations, while none was seen in the Rimifon group. However, even at this time definite evidences of toxicity were found in the Marsilid treated cultures.

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[2] Grateful acknowledgment is made to Dr. W. C. Woodard for technical assistance with this phase of the project.

After 7 days all of the remaining cultures were fixed and stained. The control group had many vessels, while the Rimifon treated series had a somewhat smaller number. Preparations having received Marsilid showed very few vessels. In the latter group there were changes suggestive of toxic injury occurring in all cells of the cultures.

The experiment was repeated but no evidence of stimulation was found when Rimifon was used at 25 γ/ml. Marsilid still proved toxic at 5 γ/ml. but at 1 γ/ml. results resembled those of the untreated controls.

By way of checking the influence of Rimifon and Marsilid on the formation of reticular fibers, explants of 9-day chick hearts in roller tube culture were studied according to the following plan:

Fluid medium	Number of explants	
	For Jacobson's stain	For reticulum stain
Marsilid 1 γ/ml.	30	30
Rimifon 5 γ/ml.	30	30
Controls (no drug)	40	40
	100	100

After 12 days' incubation all cultures were fixed. The preparations stained with both Jacobson's and the silver carbonate method showed no quantitative or qualitative difference in the cellular outgrowth and fiber formation between the treated cells and those in the controls. This at least indicated that we were working outside of the toxic range.

The experiment was repeated with leg muscle from 11-day chick embryos as a further test of the effect of Rimifon at 5 γ/ml. and Marsilid at 1 γ/ml. on mesenchymatous tissues. Series of 8 roller tubes, each containing 5 explants, were used for the two drugs and for comparable untreated controls. No significant differences were found in cultures containing the compounds under study.

Efforts to Relieve Cold Injury. Using the technique reported by Pomerat and Lewis (1953, I.), tissues from chick embryos were frozen and thawed, then mounted in plasma clots for roller tube cultures following the usual procedure. Rimifon and Marsilid were added at various concentrations in the fluid nutrient

medium. The results from this series of experiments are presented in table 1. It will be seen that experiments 30, 31, and 32

TABLE 1

The Effect of Rimifon and Marsilid on Chick Embryo Explants Following Freezing and Thawing

Expt. 10	Muscle—11 day.	Frozen to —18.0° C. in 34 secs.
	UNFROZEN CONTROL	Typical outgrowth
	FROZEN CONTROL	Not significantly different from the unfrozen control
	Rimifon	
	25 γ/ml.	Not significantly different from the controls
	12.5 γ/ml.	Not significantly different from the controls
	Marsilid	
	25 γ/ml.	Slightly injured
	12.5 γ/ml.	Slightly injured
Expt. 30	Muscle—13 day.	Frozen to —19.8° C. in 243 secs
	UNFROZEN CONTROL	Typical outgrowth
	FROZEN CONTROL	Slight inhibition
	Rimifon	
	25 γ/ml.	Like control
	Marsilid	
	5 γ/ml.	Some cultures with slightly better outgrowth than frozen controls
Expt. 31	Muscle—13 day.	Frozen to —21.0° C. in 63 secs.
	UNFROZEN CONTROL	Typical outgrowth
	FROZEN CONTROL	Slight inhibition. Fibroblasts show more damage than Expt. 30
	Rimifon	
	25 γ/ml.	Like frozen control
	Marsilid	
	5 γ/ml.	Like frozen control
Expt. 32	Muscle—13 day.	Frozen to —20.6° C. in 29 secs.
	UNFROZEN CONTROL	Typical outgrowth
	FROZEN CONTROL	Injury greater than in Expt. 31
	Rimifon	
	25 γ/ml.	Like frozen control
	Marsilid	
	5 γ/ml.	Like frozen control but occasional well-developed muscle fibers

were designed to vary the time required for freezing to approximately —20.0° C. While injury increased with a decrease in the time required for freezing, neither Rimifon nor Marsilid appeared to improve the appearance of the injured cells under the conditions of the experiment.

Cortisone Preparations

In relation to other research goals, information was accumulated on the toxicity of cortisone acetate, sulphate, and tricarballylate in tissue culture (Ruskin, Pomerat, and Ruskin, 1951). These data together with values for cortisone aldehyde and hydrocortisone are given in figure II.

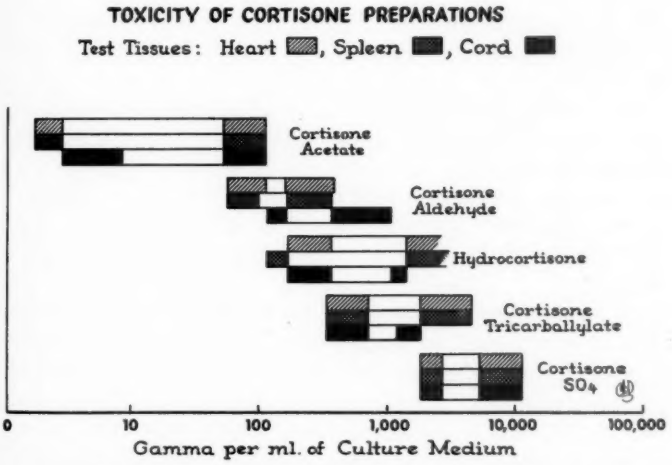

FIG. II. The left-hand block in each bar is used to represent the LID (least injurious dose) while the right-hand block refers to the MID (maximal or dose for total injury). Outgrowth like that of untreated control cultures was obtained with the value represented by the left edge while cell migration was slightly inhibited by the amount indicated by the right edge of the LID block. Almost total inhibition of outgrowth resulted from the dosage represented by the left-hand edge and no outgrowth resulted in culture containing the amount shown by the right margin of the MID block.

Due to its relatively low solubility, cortisone acetate is not easily introduced into tissue cultures without employing substances such as Tween 80 or acetone. These solvents, whose

toxicities have been described, serve to complicate experimental results. Since cortisone sulphate has little physiological effect and the tricarballylate is no longer being manufactured, effort has been directed at using the aldehyde and the hydrocortisone whose toxicities are low and therefore make them more suitable for tissue culture studies. However, the presence of preservatives in ampule preparations of cortisone aldehyde may complicate the results.

Preliminary experiments reported by Pomerat and Lewis (1954) gave evidence that a richer population of multinuclear muscle fibers was obtained from 11-day chick leg muscle frozen to —18.2° C. in 34 seconds when 10 γ/ml. cortisone acetate was included in the medium. Skin from 13-day chicks frozen to —32.0° C. in 97 seconds showed cellular outgrowth in only one out of 20 controls, but corresponding cultures containing 15γ/ml. of cortisone tricarballylate showed spindle cells in 8 out of 20 cultures.

Results obtained on the effect of adding hydrocortisone to the fluid nutrient medium used for roller tube cultures of explants exposed to cold injury at various levels are given in table 2. In all instances unfrozen tissues also received the same quantity of the drug. Both unfrozen and frozen explants without cortisone were available in each series of experiments for comparative study.

Both skin and muscle from 12-day chicks were used to obtain data in which hydrocortisone was employed at 10, 20, and 40 gamma per milliliter of the fluid medium. Results following freezing to —25.8° C. in 37 seconds shown in figure III are given for spindle cells, epithelial elements, and muscle fibers (Table 2 and Fig. IV). Untreated controls yielded poorer outgrowth in the frozen series. The addition of hydrocortisone appeared to improve outgrowth at 10 γ/ml. but the best results were obtained with 20 γ/ml. Some inhibition seemed to result from the presence of 40 γ/ml. On the basis of these observations all further work was done with 20 γ/ml. of hydrocortisone.

Figure V represents potentiometric records while table 3 and figure VI given the data obtained on 12-day chick skin and muscle frozen to —27.0° C. in 27 secs., —35.9° C. in 30 secs., and —39.8° C. in 30 secs., together with an unfrozen series.

TABLE 2

Results Obtained For 12-Day Chick Embryo Tissues in Relation to Treatment with Hydrocortisone

Expt. No.	UNFROZEN				Expt. No.	FROZEN —25.8° C. in 37 secs.			
	Skin		Leg Muscle			Skin		Leg Muscle	
Expt. 383 No hydrocortisone	Epithelium	9/20*	Muscle	19/19	Expt. 387 No hydrocortisone	Epithelium	0/20	Muscle	16/18
	Spindles	20/20	Spindles	19/19		Spindles	8/20	Spindles	16/18
						No outgrowth	12/20	No outgrowth	2/18
Expt. 384 10γ hydrocortisone	Epithelium	15/20	Muscle	20/20	Expt. 388 10γ hydrocortisone	Epithelium	6/16	Muscle	17/17
	Spindles	16/20	Spindles	20/20		Spindles	15/16	Spindles	17/17
	No outgrowth	4/20				No outgrowth	1/16		
Expt. 385 20γ hydrocortisone	Epithelium	17/17	Muscle	20/20	Expt. 389 20γ hydrocortisone	Epithelium	10/10	Muscle	17/17
	Spindles	17/17	Spindles	20/20		Spindles	10/10	Spindles	17/17
Expt. 386 40γ hydrocortisone	Epithelium	18/19	Muscle	20/20	Expt. 390 40γ hydrocortisone	Epithelium	12/16	Muscle	15/19
	Spindles	19/19	Spindles	20/20		Spindles	16/16	Spindles	17/19
								No outgrowth	2/19

*Out of 20 explants 9 showed epithelial outgrowth.

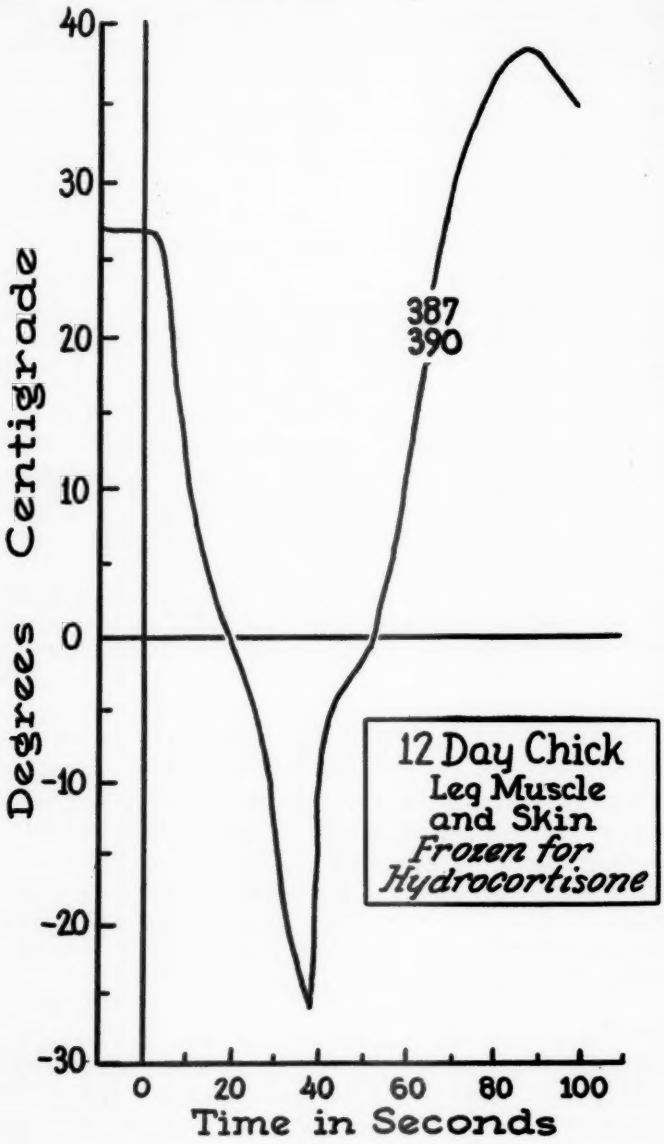
40
30
20
10
0
-10
-20
-30
Degrees Centigrade
387
390
12 Day Chick
Leg Muscle
and Skin
Frozen for
Hydrocortisone
0
20
40
60
80
100
Time in Seconds

FIGURE III

Outgrowth was not perceptibly influenced by 20 γ/ml. in unfrozen controls, but its presence generally appeared beneficial in all other series except for muscle frozen to —27.0° C. where negative results were obtained. Unfortunately, only 10 explants survived in this group compared to the 20 available in each of all other series. The injury to epithelium at —35.9° C. and —39.8° C. appeared to be so great that no recovery was obtained with the cortisone. However, at —27.0° C. no epithelial emigration occurred in the absence of the hydrocortisone but in its

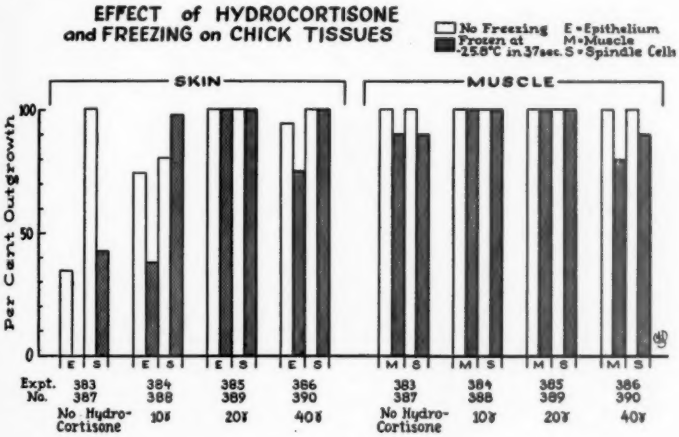

FIGURE IV

presence 25 per cent of the cultures showed outwandering cells. Muscle cells were totally inhibited at —35.9° C. and —39.8° C., but a significant number of multinuclear fibers was found in the presence of the hydrocortisone. Finally, 40 per cent of the explants showed spindle cell outgrowth in the medium containing the hormone as compared to none in its absence in the series frozen to —39.8° C.

Discussion

The effect of corticoids upon several kinds of tissues in culture has been observed by many authors. The fact that each investigator used varying quantities of different drugs and various

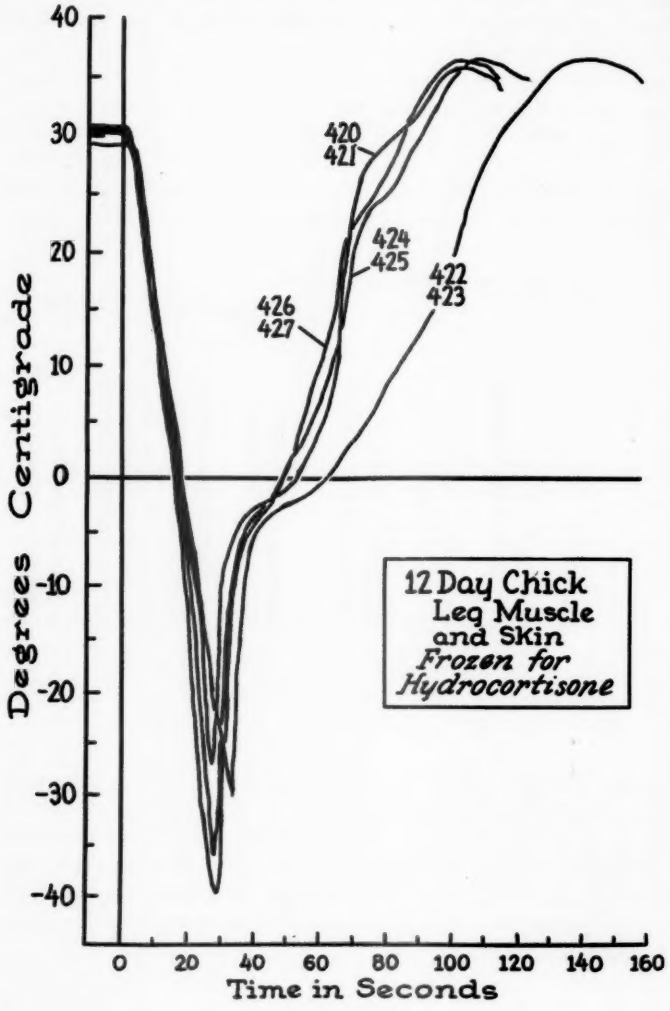
40
30
20
10
0
-10
-20
-30
-40
Degrees Centigrade
420
421
424
425
422
423
426
427
12 Day Chick
Leg Muscle
and Skin
Frozen for
Hydrocortisone
0
20
40
60
80
100
120
140
160
Time in Seconds

FIGURE V

kinds of tissues with several techniques makes it difficult to evaluate the effect of adrenal cortical extract or the individual hormones. For this reason the following results regarding each preparation are presented, although a detailed description of doses and methods of administration are omitted.

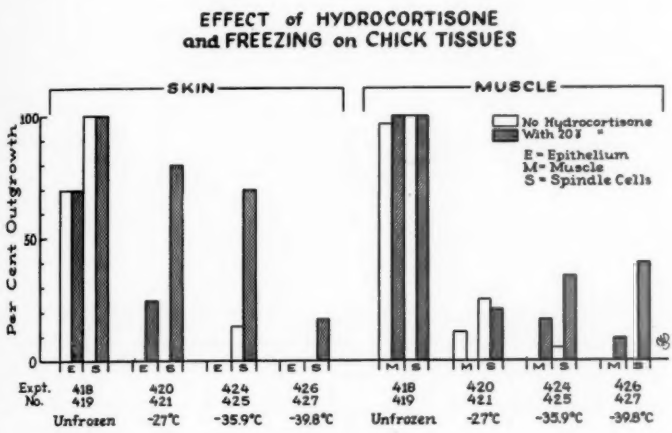

FIGURE VI

Adrenal Cortical Extract. Robertson (1948) did not observe lymphocytolysis due to A.C.E., while Feldman (1950) obtained the opposite effect with A.C.E. Schrek (1949) found that the hormone produced a delayed cytotoxic effect on thymic cells of the rabbit. The renal secretion of chick embryo renal tubules is not affected by the presence of the amorphous fraction (Chambers and Cameron, 1944).

Desoxycorticosterone. Feldman (1950) and Hechter and Johnson (1949) concluded that desoxycorticosterone does not cause any lysis of lymphocytes. Leahy and Morgan (1952) stated that desoxycorticosterone does not protect tuberculous guinea pig spleen macrophages from the direct cytotoxic action of P.P.D. Cornman (1951) found that fibroblasts were irreversibly inhibited by this hormone, whereas endothelium and the contractions of heart explants were only reversibly inhibited in tissue culture (Cornman, 1950).

TABLE 3

Results Obtained For 12-Day Chick Embryo Tissues in Relation to Treatment with Hydrocortisone

Expt. No.	Without Hydrocortisone				Expt. No.	With 20γ Hydrocortisone			
	Skin		Leg Muscle			Skin		Leg Muscle	
Expt. 418 Unfrozen	Epithelium Spindles	11/16 16/16	Muscle Spindles	17/18 18/18	Expt. 419 Unfrozen	Epithelium Spindles	13/19 19/19	Muscle Spindles	20/20 20/20
Expt. 420 —27.0° C. in 27 secs.	Epithelium Spindles No outgrowth	0/8 2/8 6/8	Muscle Spindles No outgrowth	1/8 2/8 6/8	Expt. 421 —27.0° C. in 27 secs.	Epithelium Spindles No outgrowth	3/12 10/12 2/12	Muscle Spindles No outgrowth	0/9 2/9 7/9
Expt. 422 —30.0° C. in 34 secs.	No outgrowth	10/10			Expt. 423 —30.0° C. in 34 secs.	No outgrowth	10/10		
Expt. 424 —35.9° C. in 30 secs.	Epithelium Spindles No outgrowth	0/15 2/15 13/15	Muscle Spindles No outgrowth	0/17 1/17 16/17	Expt. 425 —35.9° C. in 30 secs.	Epithelium Spindles No outgrowth	0/11 7/11 4/11	Muscle Spindles No outgrowth	2/16 4/16 12/16
Expt. 426 —39.8° C. in 30 secs.	No outgrowth	20/20	No outgrowth	20/20	Expt. 427 —39.8° C. in 30 secs.	No outgrowth	20/20	Muscle Spindles No outgrowth	2/20 8/20 12/20

Desoxycorticosterone Acetate. Hechter and Johnson (1949) recognized the fact that this drug does not promote lymphocytolysis.

Cortisone (11-dehydro-17-hydroxycorticosterone, Kendall's Compound E). Baldridge, Kligman, Lipnik, and Pillsburg (1951), Hechter and Johnson (1949), and Feldman (1950) found that cortisone has no inhibitory effect upon the cellular elements of human buffy coat or on lymphocytes from other sources. However, Paff and Stewart (1953), Leahy and Morgan (1952), and Heilman (1945a) came to the conclusion that this hormone inhibits the activity of wandering cells and causes the degeneration of some lymphocytes. On the other hand, some enhanced activity or outgrowth with the administration of cortisone has been shown for fibroblasts by Baldridge, Kligman, Lipnik, and Pillsburg (1951), for macrophages by Heilman (1945b) and in renal secretion by Chambers and Cameron (1944). Paff and Stewart (1953) observed that sometimes the hormone inhibits the fibroblasts and at other times has no effect on fibroblasts.

Cortisone Acetate. Holden and Reiman (1951) treated mammalian leucocytes and fibroblasts with cortisone acetate, while Leahy and Morgan (1952) used it on macrophages. Both groups concluded that this preparation inhibits the activity of the test tissues. Steen (1951), using 25 times the therapeutic dosage of cortisone acetate, found no inhibition of the outgrowth of chick embryo heart muscle, lung and intestine.

11-dehydrocorticosterone (Kendall's Compound A). According to Hechter and Johnson (1949), this hormone does not promote lymphocytolysis, while Feldman (1950) found the reverse effect. The degeneration of small and medium sized lymphocytes in the presence of Compound A has also been shown by Heilman (1945a). Moreover, Heilman (1945b) found that macrophages are stimulated with the same hormone, although he pointed out that this may be due to the damage of other cells and not to a direct effect of the drug itself. Chambers and Cameron (1944) found this preparation to have no effect on renal tubular secretion *in vitro.*

Corticosterone. According to Hechter and Johnson (1949), this hormone does not exhibit lymphocytolytic activity. Schrek

(1949) came to the conclusion that corticosterone decreased the viability of thymic cells.

Hydrocortisone (17-hydrocorticosterone, Kendall's Compound F). Leahy and Morgan (1952) observed that this compound inhibits the cytotoxic effect of P.P.D. on the macrophages of tuberculous guinea pig spleen and Schrek (1949) in treating the thymic cells of the rabbit with hydrocortisone found that it had a cytotoxic effect.

It will be seen from this brief review of literature that data obtained with cell culture techniques have led to divergent conclusions. Our own work showed strikingly better results when hydrocortisone at 20 γ/ml. of the nutrient medium was added to cold injured explants, but this effect was not sufficiently clear to bring conclusive proof to settle this knotty problem. However, in view of the importance of finding chemical agents which may assist in the mechanism of cellular repair, it is believed important to make our data available to other workers.

Summary

Rimifon and *Marsilid* which had been suspected of being general stimulants of connective tissue failed to produce better outgrowth from cold injured explants than corresponding untreated controls. Some evidence was found for the beneficial action of *cortisone*, particularly hydrocortisone at 20 γ/ml. of nutrient medium, in stimulating cellular recovery from cold stress.

We are indebted to Mr. McDonald Smith for the preparation of the graphs in this series of reports.

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IMPROVEMENTS IN LETTER STYLES FOR SIGHT-TESTING CHARTS*

J. H. PRINCE

The subject of sight-testing typography has received almost continuous attention during the last seventy-five years. Because the need for improved sight-testing charts has always been urgent, ophthalmologists and optometrists too have quested constantly for fresh data. But most of the experimental work undertaken has lacked the benefit of the technical knowledge and equipment which have more recently become accessible.

We know now that the presence of astigmatism in an eye varies the image of a letter by altering the thickness and length of its limbs. Even when astigmatism is absent there are points of confusion where the limbs of a letter join. We find this particularly in 'R' and 'K'. There are indeed very few letters entirely free from this defect.

In 1888, Sanford carried out experiments with lower case letters in Snellen type, and he decided, as so many other workers have done, that each letter takes a particular place in order of legibility. In other words there is a percentage threshold of recognition for every letter and numeral used in print and in sight-testing charts. For instance the letter 'T' can be seen at a distance 47 per cent greater than the letter 'B', so that the letter 'T' can be relatively smaller than the 'B', and when viewed at the same distance they will then be equally legible.

What is important too, is that Sanford also found he could alter the sequence of legibility completely by varying the letter style. Thus he was one of the first to establish the need for a standard style, something which is still not achieved sixty-six years later. This is important because without a standard style, no two ophthalmologists can be sure that their visual-acuity ratings coincide on any particular patient or group.

Jackson, whose work is well known, decided around 1914 that refraction and the measuring of visual acuity demand different symbols or letter-styles, and that what may be best for the one,

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may not be so suitable for the other. We not only concur with this, but intend to give it attention very shortly.

At about the same time, Barbara Roethlin used a mobile chart on a track to test the relative legibility of the letters of the alphabet in fifty-two different faces of type. But the utility of her findings was confined to near-vision testing. She did however verify the suitability of letters which are as wide as they are high, which is in keeping with the general practice of forming sight-testing letters within a square.

Jackson soon gave more attention to this subject, and he produced evidence to support the belief that non-serifed letters can be recognized at distances fifty per cent greater than serifed letters such as Snellen used. At the meeting at which Dr. Jackson produced this figure, Dr. Nelson Black roused curiosity regarding the effects of astigmatism at various axes on the breaks in Landolt rings, something which is now receiving the attention it deserves, although we have recently discovered that even a person with normal vision will find the visual threshold of a Landolt ring differs with the angle at which the break is placed.

In 1937, I experimented in England with variable recognition-thresholds, and produced tables of letter sizes for every letter and each normally accepted testing distance, which were then incorporated into two prototype charts on which approximately twenty thousand refractions were successfully completed. The letters were standard face without serifs, and each limb was twenty per cent of the width of the entire letter. Now it has been found possible to improve the accuracy of these figures, and the values obtained in experiments at this hospital are included in this article.

It is certain that most standards in use at present take into consideration too few of the factors concerned with the human or psychological aspects of vision, and which are less easily controlled. Present standards give all attention to the mechanical aspects which usually *can* be controlled, and tend to ignore the fact that even if psychological factors cannot be controlled easily, they can be accommodated more than they have been in the past.

In the experiments carried out in this department, a track fifteen feet long (Fig. I) was installed in a thirty-foot room. On this track, which was at eye level to a seated subject, a dolley ran by remote control, either forwards or backwards at a speed of

five feet a minute. On the dolley, a lamp-house and film winders were mounted so that a film which had black letters on a white ground, and also white letters on a black ground printed on it, all uniform in size, could be used to show one illuminated letter at a time. The letter size was 5.5 mm., and the width of each limb was 1.1 mm.

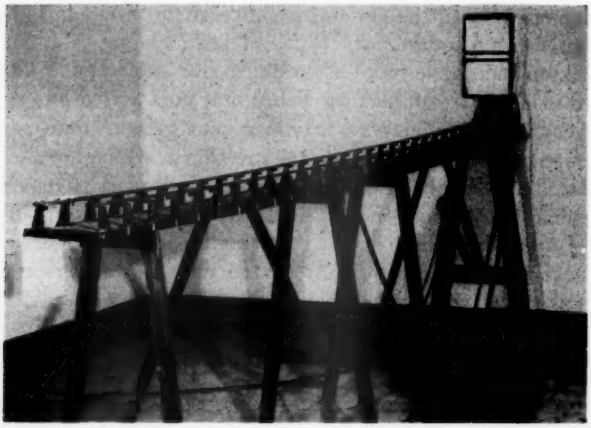

FIG. I. The fifteen-foot track with mounted dolley and chart.

Starting from the maximum distance, this could be moved forward by the observer to the position of the recognition threshold; and the procedure could be repeated for every letter of the alphabet and every numeral. In this way, an accurate distance from the eye at which the letter first became visible could be obtained, and relative distances between all the letters and numbers found.

When this apparatus had been in use for a while, it was modified for the following reasons. It was found that a small target of only one letter created rapid retinal fatigue because of the restricted area of stimulation, and that the speed at which the subject became fatigued inhibited the usefulness of the test beyond the initial stages. In order to overcome this, the room was lighted diffusely around the installation, and a flasher introduced into the lighting circuit of the projector to halve the time of retinal stimulation.

Different qualities of light were also experimented with in an effort to eliminate this fatigue, but ultimately it was found to be so rapid and so great that it would have been unwise to accept any of the experimental findings. It was only by stimulating a large area of retina that this difficulty was overcome. So the single letter projector was replaced with an internally illuminated chart 14 in. × 9 in., with an opal plastic panel giving an even illumination of 7 ft. Cs., and built to accept a sliding panel of letters which could be removed and replaced by another instantly.

The letter size of 5.5 mm. was retained, and photographed letters and numerals mounted on the slides, thirty-four in all, but never more than six on a slide. The grouping of these letters and

FIG. II. The remote control, held by the observer. When the contact is pressed on one side the dolley moves forward, and when pressed on the other, it moves backwards.

numerals was carefully arranged to produce a minimum of confusion and a minimum of suggestion.

The subject found the recognition threshold of each letter and numeral by moving the chart forward with a remote control, and positioned for each one were tabulated in this way. Frequent rests were imposed throughout the proceedings.

Although fatigue appeared to be no longer the hazard it had been before the apparatus was altered, it was felt that its possible effects should be eliminated from the calculations to the greatest extent, and with that idea, each subject was tested a second time with the order of the letters exactly reversed, and after a lapse of several days between the tests. From these two sets of readings, an average of considerable reliability could be obtained.

When approximately three thousand readings had been taken in this manner, the results were analysed provisionally to see if any modification of the experiment was necessary before proceeding further.

Many of the letters appeared to be easily confused just prior to reaching the visual threshold, and when considered with numerals also, there were twenty-one pairs which produced some kind of confusion. The most important of these were 'C' and 'G', 'O' and 'Q', 'X' and 'K', 'S' and '5', 'S' and 'B', 'V' and 'Y', 'Y' and 'T', 'W' and 'V', '6' and 'R'.

Whilst some of these confusions are understandable because of the shapes of the letters, some are rather mystifying, and are obviously the result of non-visual factors. These findings readily verified that the deciding factor in the recognition of many letters is the relationship between the amounts of black and white in the letter squares. In a 'W' or an 'M' for instance, the amount of black in the square is much greater than in an 'L' or a 'T'. The same thing applies to a 'B' or an '8'.

Other letters were made therefore in which areas of confusion where limbs join each other, were reduced by tapering the limbs at their insertions into other limbs (Fig. III). This not only reduces the amount of black in a given area, but also reduces the amount of black in relation to white in the whole letter square.

In addition to these letters, others were designed with limbs subtending an angle of 0.8′ of arc to the eye, whilst the whole letter retained a 5′ arc angle. Others were made too, which had

breaks in each limb at the points of junction with other limbs, each break being equivalent to the angle subtended by the width of the limb.

FIG. III. The tapered letters in which the area of blackness is reduced where the limbs join.

The broken letters appeared to have no advantage over the standard ones, but the thin ones and the tapered ones did. The thin ones were contrasted with the standard ones first, when ten specific letters were chosen for re-designing in such a manner that we could discover by what features some letters are recognized. For instance, is a 'P' recognized more readily because of the size of its stem, or of its loop? Theoretically it should be from a combination of all features, but this does not always appear to be so. Then too, is an 'A' recognized by the point at the top, or by the height of its horizontal member? How long must the lower stem of a 'Y' be to avoid confusion with a 'V'?

The ten letters chosen were 'K, Y, B, M, R, W, G, S, P, A'; and of these 'K, Y, B, M, R, and W' were each produced in five forms, all non-serifed. They were: 1. A standard square 5′ letter with limbs subtending 1′ arc; 2. The same as above but with the limbs subtending 0.8′ arc; 3. The same as No. 1 but with the limbs tapered in the regions where they joined each other; 4. A modified form with the dimensions of No. 1; 5. A modified form with the dimensions of No. 2.

The modifications were as follows: In 'K' Nos. 1 and 2 were repeated but with the two angled limbs meeting together at a point exactly halfway down the vertical limb. In 'Y' Nos. 1 and 2 were repeated but with the junction of the three limbs at a point three-eighths of the height of the letter instead of half-way up.

In 'B' Nos. 1 and 2 were repeated but with the centre limb raised 5 per cent of the letter height. In 'M' the point of the centre limbs was raised 40 per cent of the height of the letter. In the 'R', Nos. 1 and 2 were repeated with shorter tails and stems, giving the loop a 50 per cent increase in height. 'W' was produced with a slightly broader base, thus decreasing the angle of the outer limbs from the vertical. The difference made in 'G' was the addition of a tail. In 'S' the neck was made horizontal as well as diagonal, and in 'P', as in 'R', the stem was shortened by giving the loop an increase in height of 50 per cent.

FIG. IV. The other non-tapered letters used to produce the table of findings given in the text.

The 'A' was treated slightly differently. No. 3, with limbs subtending an angle of 0.8′ arc had its horizontal limb lowered to the amount of 50 per cent of its thickness, and this reduced the lower aperture by 25 per cent. No. 4, the limbs of which subtended 1′ arc not only had this feature, but also had its top point increased in width by 200 per cent.

The Results of taking fresh readings with these newly designed letters were somewhat startling although not altogether unexpected. Early in the experiments it was noticed that in some letters in which the amount of black in relation to white in the letter square was greatest, the thin letters were preferred to the standard face letters. Of the ten specially designed letters this applied to 'B, M, R, W, G, S'. But these findings had to be abandoned completely as soon as letters with tapered limbs were introduced, for in several instances, notably in 'K', 'Y', and 'R', there was an immediate and complete switch of preference from thin to

tapered forms. The way in which the letters were chosen is illustrated in the following table, which demonstrates those ten letters specially designed.

FIG. V. The Snellen form of letters contrasted with the improved form designed in these experiments.

It will be seen from the foregoing table that the tapered letters were picked very unanimously in some instances, but in 'W' the choice was shared between the thin standard and the thin one with a broader base, so a compromise between these two must be adopted for future experiments. In 'A' the choice was shared by the thin form with a lower horizontal limb, and the 1′ arc letter with a lower horizontal limb and a broader top, so again a compromise is demanded. All other letters were left in the standard 1′ arc non-serifed form, so that the final alterations from the standard form will be confined to 'K, Y, B, M, R, W, G, and A'.

Letter	Design	Percent subject preference		
		Total percent	First percent	Second percent
K.	Standard 0.8′ arc	6	0	6
	Standard 1′ arc	68	22	46
	Tapered limbs	*84*	*64*	20
	0.8′ arc limbs meeting in centre..	20	2	18
	1′ arc limbs meeting in centre....	22	12	10
		200	100	100

The analysis also demonstrated that some observers make their two choices on the basis of form, whilst others make it on the basis of limb thickness, something that may be controlled by the distribution of retinal cones. Still other observers confuse these two

factors and choose some letters because of form, and the remainder because of thickness or the amount of blackness. For instance, they may make their first choice of a letter as the thin standard form, and their second choice as a standard thickness but a modified form. In most cases however, the percentage of observers choosing a particular letter permitted no doubt as to the certainty of majority choice. In the same way it was possible to reject some letter forms at once, and without any doubt.

Letter	Design	Percent subject preference		
		Total percent	First percent	Second percent
Y.	Standard 0.8′ arc	22	6	16
	Standard 1′ arc	46	16	*30*
	Tapered limbs	*82*	*62*	20
	0.8′ arc short stem	24	8	16
	1′ arc short stem	26	8	18
B.	Standard 0.8′ arc	24	10	14
	Standard 1′ arc	40	12	*28*
	Tapered limbs	*56*	*36*	20
	0.8′ arc raised centre limb	46	32	14
	1′ arc raised centre limb	34	10	24
M.	Standard 0.8′ arc	62	30	32
	Standard 1′ arc	54	20	*34*
	Tapered limbs	*70*	*44*	26
	0.8′ arc raised centre limbs	12	6	6
	1′ arc raised centre limbs	2	0	2
R.	Standard 0.8′ arc	22	4	18
	Standard 1′ arc	80	6	*74*
	Tapered limbs	*96*	*90*	6
	0.8′ arc larger loop	0	0	0
	1′ arc larger loop	2	0	2
W.	Standard 0.8′ arc	*60*	*30*	*30*
	Standard 1′ arc	42	14	28
	Tapered limbs	42	24	18
	0.8′ arc, wider base	54	*30*	24
	1′ arc, broader base	2	2	0
G.	Standard 0.8′ arc, no tail	68	32	*36*
	Standard 1′ arc, no tail	30	16	14
	Standard 0.8′ arc with tail	*80*	*50*	30
	Standard 1′ arc with tail	20	2	18
S.	Standard 0.8′ arc angled neck	52	16	*36*
	Standard 1′ arc angled neck	*76*	*52*	24
	Standard 0.8′ arc horizontal neck	56	30	26
	Standard 1′ arc horizontal neck	14	2	12
A.	Standard 0.8′ arc	42	24	18
	Standard 1′ arc	24	4	20
	0.8′ arc, lower horizontal limb	*72*	18	*54*
	1′ arc, lower horizontal limb, etc.	62	*54*	8
P.	Standard 0.8′ arc	52	12	*40*
	Standard 1′ arc	*74*	*48*	26
	0.8′ arc, larger loop	34	26	8
	1′ arc, larger loop	40	14	26

Verification that the chosen forms of letters have advantages over Snellen letters was demonstrated photographically by taking pictures of both kinds under simulated conditions of myopia and astigmatism, and the results are shown in figures V to XII. Not only were the new letter forms found to be justified, but it was also decided that further consideration would have to be given to designing letters in two specific forms, one for carrying out refraction, and the other for measuring visual acuity, or alternatively a compromise form as nearly suitable for both purposes as possible; for not all letters of the alphabet are equally suitable for both purposes.

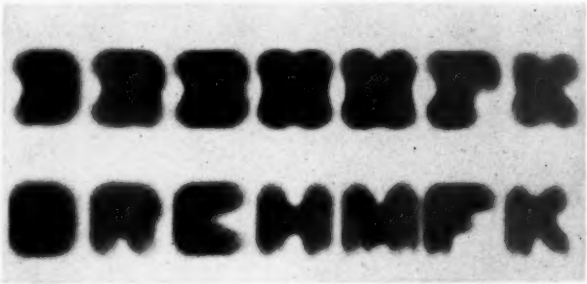

FIG. VI. The same letters as figure V photographed with simulated high myopia.

Finally, it was decided to try to eliminate the roundness that the limbs of letters take on in high visual errors, the ends appearing spherical instead of square. This was done by extending the

FIG. VII. The same letters photographed with simulated astigmatism with the greatest error at 90°.

FIG. VIII. Letters 'E' and 'R' in Snellen and improved types, with simulated high myopia.

FIG. IX. The same as figure VIII with astigmatism with greatest error at 180°.

FIG. X. The same as figure IX with astigmatism with greatest error at 90°.

points or corners slightly as shown in figure XI, and this did correct the defect to some degree.

FIG. XI. The improved letters with extended corners.

Summarising this first step of a long project it can be said that we have produced a type-face for optotypes which, because of variations in form and limb thickness, will give a maximum recognition factor, and a minimum of confusion. The number of readings taken in our experiments so far is four thousand, and with the design of letters and numerals once perfected, we shall

proceed to repeat our experiments on visual thresholds, and produce an accurate table of thresholds for every letter and numeral in the new forms.

FIG. XII. The same letters as in figure XI, but with simulated astigmatism with greatest error at 90°.

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SMALL MOTION TRANSDUCER FOR RADIOBIOLOGICAL RESEARCH

EVERETT O. RICHEY AND HERBERT B. GERSTNER*

In the development of equipment for studying the effects of high intensities of ionizing radiation on muscles, the problem of recording small isotonic motions was encountered. Since it was necessary for the operators to be protected, the recording system had to be at a safe distance from the source of radiation. Thus, the use of a simple and dependable transducer was imperative. Such a device was obtained by employing a potentiometer recently developed for telemetering and control of guided missiles. It proved to be very satisfactory and so flexible in application that a brief description should be of general interest.

Technical Problem. Both the resting length and the isotonic contraction curve of all three types of muscle tissue—skeletal, heart, and smooth muscle—had to be recorded; thus, the motion encountered varied in speed from 0 to about 100 cm./sec. Therefore, the transducer had to transmit constant potentials as well as rapidly changing transients. The optimal load for the different muscle preparations ranged from 5 to 500 gm. To obtain a distortion-free recording throughout this load range, the force required to operate the transducer had to be small compared to 5 gm. During muscle contraction, the displacement of the load extended from 0 to 10 mm. This displacement had to be measured with an accuracy of about 0.1 mm.

Some of the experiments involved prolonged exposure of the test muscle and the transducer to irradiation of high intensity, thus excluding adjustment of the instruments by personnel. Therefore, the transducer had to show utmost electrical stability, even though exposed to ionizing radiation, strong electric fields (X-ray machine), and fluctuations in line voltage. Furthermore, a large and linear output signal was considered advantageous to eliminate instabilities inherent in multistage, high gain, D.C. amplifiers, and to simplify interpretation of recordings.

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Most of the transducers commonly used in muscle physiology do not meet the above-stated requirements. Strain-gage, electromagnetic, and crystal transducers must be discarded because of their inability either to transmit direct current or to record isotonic motion. Mechanical and optical systems also must be excluded because of their failure to permit a sufficient distance between transducer and recorder. Photoelectric devices were found too sensitive to ionizing radiation to be practical. Thus, it became expedient to examine the suitability of a potentiometer as an electromechanical transducer under the given conditions.

Characteristics of the Potentiometer

The experiments were performed with a single-turn wire-wound potentiometer—the Tinytork, Model T.[1] This potentiometer is shown in actual size in figure I. Its outstanding characteristics are: (a) a shaft supported on two

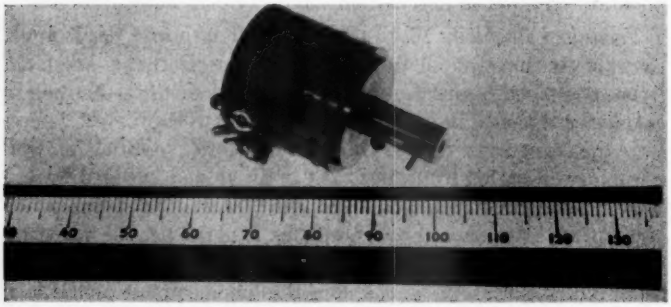


FIG. I. Helipot potentiometer Tinytork, Model T, with pulley and pin attached (scale in millimeters).

ball bearings to eliminate radial side play; (b) a 360-degree continuous mechanical rotation and a 355-degree electrical rotation; (c) a starting torque of approximately 0.4 cm. gm. and a running torque of negligible value; (d) a moment of inertia of 0.0004 gm. cm.^{2}; (e) a resistance of 50,000 ohms with linearity tolerance of $\pm$ 0.5 per cent. To judge the acceptability of a potentiometer for a specific purpose, the effect of four main sources of inaccuracy—resolution, noise, nonlinearity, and loading—must be evaluated.

The resolution of a wire-wound potentiometer is defined as the reciprocal of the number of turns of wire. When the sliding contact moves along the coil, the voltage changes in finite steps, the magnitude of which is determined by the resistance of each individual turn. Consequently, a decrease in resolution error is obtained by an increase in the number of turns of wire on the coil.

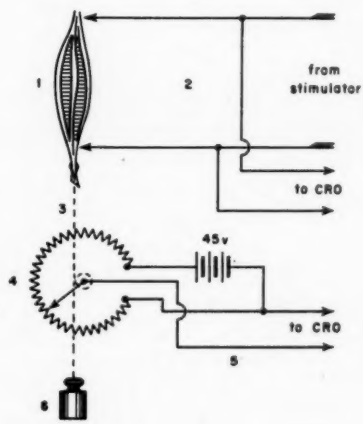
[1] Helipot Corporation, 916 Meridian Ave., South Pasadena, Calif.

The noise of a wire-wound potentiometer is composed of spurious signals originating during slider movement. Such movement results in changes of contact resistance, variations of contact potential, and slight deviations from linearity caused by the resolution error.

For an ideal linear potentiometer, the graphic representation of the slider voltage plotted against the shaft position is a straight line. Under practical conditions, however, certain deviations from ideal linearity are inevitable. The maximal deviation of the slider voltage from a straight line, expressed in percentage of total coil voltage, is commonly referred to as linearity tolerance.

The loading error of a potentiometer is the divergence from linearity that is caused by current flow through the slider contact. Any finite external load resistance causes such a flow and, thereby, brings about a disturbance of the linear potential distribution along the potentiometer coil. This disturbance will be small when the external load resistance is large compared to the total coil resistance. The properties of the Tinytork—so far as resolution, noise, and linearity are concerned—will be presented under "Discussion."

Examples of Practical Application. Figure II shows the essential features of an experimental arrangement designed for the study of isotonic, skeletal muscle contractions. The silk thread. connecting the tendon of an isolated frog gastrocnemius to a load



1. MUSCLE
2. STIMULATING ELECTRODES
3. THREAD
4. POTENTIOMETER
5. TRANSDUCER CIRCUIT
6. LOAD

FIG. II. Experimental arrangement used for study of skeletal muscle contractions.

of 50 gm., was wound around a pulley of 6.3 mm. diameter which was solidly attached to the potentiometer shaft. To prevent slippage, the thread encircled a pin projecting radially from the pulley. Since the moment of inertia and the starting torque of the rotating system were extremely small, the friction between thread, pulley, and pin sufficed to insure good mechanical connection. Under the conditions described, the efficiency of the transducer was as follows: With a pulley circumference of approximately 20 mm. ($\pi \cdot 6.3$ mm.), a muscle movement of 1 mm. caused a shaft rotation of 18 degrees. This rotation was accompanied by a change in slider voltage amounting to $\frac{18}{355} = 0.0507$ times the total coil voltage. In the present experiment, 45 volts were applied to the potentiometer; therefore, a muscle movement of 1 mm. produced a potential change of 2.3 volts. By the use of a four-channel, D.C., cathode ray oscilloscope (CRO) in combination with a camera as the recording device, a thorough study of

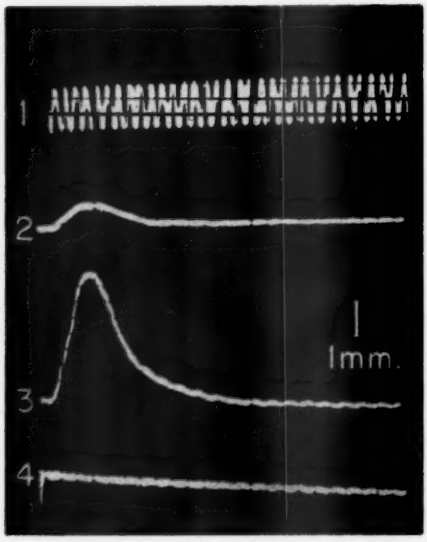


FIG. III. Four-channel cathode ray oscilloscope record: *1*, 100-c.p.s. time marker; *2*, isotonic contraction of isolated and irradiated frog gastrocnemius; *3*, isotonic contraction of nonirradiated gastrocnemius isolated from same frog; *4*, stimulus, a condenser discharge.

the slider potential and, thereby, of the muscle movement was possible.

Figure III is a typical example chosen from an experimental series in which the effect of ionizing radiation on the skeletal muscle was studied. Application of a common sweep to the electron beams resulted in identical time axes for all four curves. The uppermost tracing, *1*, indicating intervals of 10 msec. from one spike to the next, was obtained by feeding a 100-c.p.s. sine wave into one of the oscilloscope channels. Curves *2* and *3*, representing isotonic contractions of gastrocnemii isolated from the same frog, were recorded from the output of two Tinytork potentiometers. One of the muscles (*2*) had been exposed to 60 kr. of x-radiation, while the other (*3*) served as a nonirradiated control. The downward spike at the left end of tracing *4* was produced by the stimulus—a condenser discharge. The curves in figure III permit a rapid and accurate analysis of isotonic skeletal muscle contractions. Without difficulty, the characteristic factors—time of latency, amplitude, duration, and speed of motion—can be obtained directly from the record.

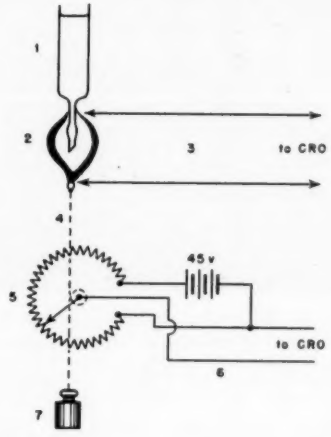


FIG. IV. Experimental arrangement used for study of electrical and mechanical activity of isolated frog heart.

The experimental arrangement shown schematically in figure IV was designed to study the effect of ionizing radiation on mechanical activity and action potential of isolated frog hearts. The tip of a glass cannula, introduced into the ventricle through the aorta, supplied the heart with Ringer's solution. A silk thread connecting a load of 5 gm. to the apex of the heart was attached to the potentiometer in the way described above. Pulley size and coil voltage also were the same as used for the skeletal muscle. In addition, two wire electrodes touched the base and the apex of the heart. By recording the potential difference between these electrodes together with the slider potential of the Tinytork, simultaneous curves for action potential and mechanical activity were obtained.

Figure V represents two typical examples of recordings, both obtained from the same heart with the film speed in section B

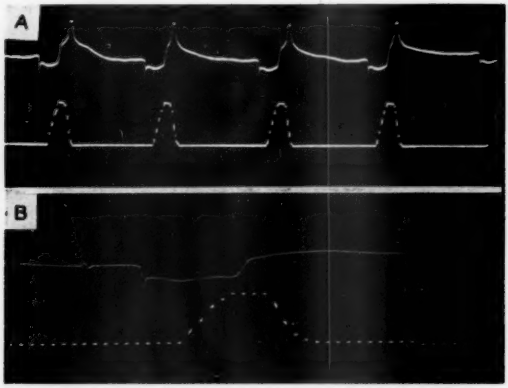


FIG. V. Two-channel cathode ray oscilloscope record of isolated frog heart. *A*, Low-speed recording. *B*, Recording at five times greater speed than *A*. In each section the upper trace is the electrocardiogram; the lower trace represents the mechanical contraction interrupted by 40-msec. Time marks.

amounting to five times that in section A. The upper curve in each section is a reproduction of the action potential. The lower curves are a record of the heart motion as indicated by the Tinytork. By modulating the intensity of the CRO beam with a sine wave signal of 25 c.p.s., a periodic interruption of the trace was obtained with a time interval of 40 msec. between consecutive

spots. The recordings thus produced allowed an analysis of the mechanical and electrical activity of isolated hearts.

To eliminate recording errors, a CRO with camera was used in the two examples described above. For many purposes, however, a simpler recorder may be employed as demonstrated by the following experiment: An isolated frog heart, attached to a load of 5 gm. and the Tinytork, was placed at a distance of 10 cm. from the target of a 260-KVP Picker X-ray unit and subjected to a dose rate of 6 kr. per minute. Two leads connected the potentiometer to an inkwriter which was located outside the X-ray room. Even though not protected against radiation and electrical fields, the Tinytork produced a record free of distortion.

Discussion. From results of experiments in which a Tinytork potentiometer was used as an electromechanical transducer, the following conclusions can be drawn: (a) The noise level of the transducer was less than the measuring accuracy of ± 0.1 volts, even with the high speed encountered in skeletal muscle contractions (Fig. III); (b) the starting torque and the moment of inertia of the transducer did not introduce appreciable errors, even with a small mechanical load of 5 gm. (Fig. V); (c) the resolution error of the transducer was less than the measuring accuracy of ± 0.1 mm.; and (d) the radiation sensitivity of the transducer was so small that the highest dose rate used (10 kr. per minute) produced no measurable effect. The above-stated conclusions indicate that the Tinytork potentiometer can be used as an effective isotonic motion transducer.

Summary. A potentiometer has been used as an electromechanical transducer in muscle studies. Undistorted recordings of isotonic contractions were obtained, even under conditions of high speed, low force, and high radiation intensity. Experimental examples of practical applications show the suitability of the potentiometer for investigating the effects of x-radiation on muscle twitches and for studying the correlation of mechanical contraction and action potential in the heart.

ON THE NATURE OF DISEASE*

HANS SELYE

The title I have chosen for this Meyer Bodansky Memorial Lecture is undoubtedly presumptuous. However, it is perhaps fitting to think about fundamental problems in medicine, at an occasion such as this, when we wish to honor this great biochemist who has done so much to clarify our views on the chemical basis of disease.

In several of my books on stress (Selye, 1950, 1951, 1952; Selye and Horava, 1952, 1953), I had attempted to use the adaptation syndrome concept as a basis for a sketch of a unified theory of medicine. Of course, any attempt at a perfect unification would be *a priori* doomed to failure since the various diseases differ both in their pathogenesis, and in their manifestations, hence, they could never be reduced completely to any one common denominator. Nevertheless, efforts to arrive at a unified concept of disease have been made by physicians ever since the beginning of medical history. Whenever objective observation revealed any one structural or functional characteristic to be common to all the parts of the body, or to all of its diseases, efforts were made to single out this characteristic as a look-out post from which to obtain a co-ordinated view of medicine as a whole.

If we do not go any further than this, if we do not look upon these constructions of the mind as anything more fundamental than an elevated position from which we can get a synoptic view of disease, such efforts are, to our mind, quite permissible.

In our "First Annual Report on Stress" (Selye, 1951) where we attempted to explore possibilities in this direction, we expressed our views as follows:

"Whenever a large number of facts accumulates concerning any branch of knowledge, the human mind feels the need for some unifying concept with which to correlate them. Such integration is not only artistically satisfying, by bringing harmony into what appeared to be discord, but also prac-

* From the Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada. The 1953 Meyer Bodansky lecture at The University of Texas Medical Branch, Galveston, sponsored by the Phi Delta Epsilon Medical Fraternity.

tically useful. It helps to see a large field from a single point of view. When surveyed from a great elevation some details in the landscape become hazy, or even invisible; yet, it is only from there that we can see the field as a whole in order to establish where more detailed exploration of the ground would be most helpful for its further development."

Stress plays an important part in many diseases. Its systemic manifestations, the general adaptation syndrome (G-A-S) (Selye, 1950, 1952) and its regional expressions, the local adaptation syndrome (L-A-S), encompass the very essence of what we call disease. It was rather tempting, therefore, to use the concept of stress for some degree of unification.

Summary of Earlier Views on Stress and Disease

In the earlier volumes of this series, we developed our concept of disease to a point which has been illustrated by the following schematic drawing.

All *agents*, which act upon the whole body or any of its parts *(target)*, exert dual effects:

(1) Specific actions *(interrupted arrow)*; for instance, ether causes anesthesia, Salmonella typhi produces typhoid fever.

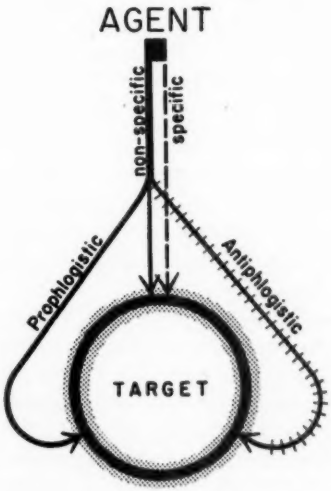


FIGURE 1

(2) A non-specific stereotypical reaction of stress, which manifests itself in the form of the general adaptation syndrome *(three-branched arrow)*.—Stress affects the target directly. The topical tissue injury caused by a burn, the immediate effects of microbes upon cells are cases in point *(straight branch of solid arrow)*, but some of its actions are mediated through humoral or nervous pathways *(curved branches of this arrow)*.

The principal humoral pathways lead through the anterior pituitary and the adrenal cortex, which produce essentially "adaptive" hormones. Among these, ACTH and the *antiphologistic*-corticoids (*e.g.*, cortisone) inhibit, STH and the *prophlogistic*-corticoids (*e.g.*, DCA) stimulate inflammatory responses to injury.

The most prominent reaction-patterns to topical stress are inflammation and necrosis, to systemic stress, "shock." Various combinations of these basic responses constitute the essence of most diseases.

The regulation of tissue reactivity, for instance through adaptative hormones, often determines whether the body succumbs to disease or resists a potential pathogen by means of adaptation. Other humoral agents and the nervous system also participate in the adaptation syndrome, but the nature of their pertinent actions is not yet sufficiently understood to permit a detailed consideration at the present time.

All the effects of biologic agents, and particularly those of the adaptive hormones, largely depend upon "conditioning factors" *(shaded area)*, some of which are external (diet, temperature, light), others internal (heredity, constitution, previous exposure).

Thus a state of stress is produced in the body through multiple pathways, its effects being always modified by the variable specific actions of the eliciting agent and by the conditioning factors (the "constitution" or "terrain") which are different in every individual, in every part of the individual. These factors can affect target and pathway selectively, and thereby vary the manifestations of what, in essence, is but a single stress-response.

In this way, a somewhat more unified concept of pathology began to take shape. The most varied manifestations of disease were considered to have a *tripartite pathogenesis*, consisting of: (1) the direct action of the exogenous agent, (2) endogenous factors, which inhibit this action, and (3) endogenous factors, which facilitate this action.

We must always analyze each of these three components; it is not enough to diagnose a condition as inflammation and to prescribe an antiphlogistic hormone if we wish to use this concept as a guide to therapy. It may be apparently quite logical to treat a case of say, acute appendicitis or of tuberculosis with cortisone, because these are manifestly inflammatory lesions and the hormone is an anti-inflammatory one. However, an analysis of the entire pathogenic situation can prevent such grave errors.

It will show that, in these instances, inflammation is a useful phenomenon, necessary to put up local resistance to the otherwise highly damaging topical stresses, the microbes. Here the principal pathogenicity of the evocative micro-organisms does not lie in any tendency to elicit excessive inflammation. On the contrary, only too often they do not produce a sufficiently strong inflammatory barricade to prevent their tissue-damaging effects and their spread throughout the body.

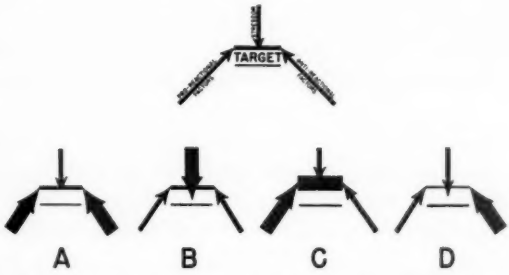


FIG. II. *Schematic illustration of relationship between target, stressor, pro-reactional and anti-reactional factors.* The top diagram shows the basic relationships under standard conditions. The stressor is directed against the target, but does not actually penetrate into it here, since it is not strong enough to overcome the resistance of the target's reactive surface (*e.g.*, an inflammatory barricade). *A*. Both pro- and anti-reactional factors are excessively developed, hence the condition of the reactive surface remains unchanged. The mild stressor again fails to penetrate into the target. *B*. Pro- and anti-reactional factors (and hence the reactive surface) are normally developed, but the stressor is of excessive intensity, so that it penetrates into the target. *C*. Pro-reactional factors are overabundant and therefore the reactional surface hypertrophies in excess of the stressor's requirements; consequently, the latter is far from penetrating into the target. *D*. Anti-reactional factors are unduly active, hence the reactional surface is so weak that even the mild stressor readily penetrates into the target.

[The granuloma-pouch can serve as an experimental model in which all these relationships have actually been verified. In the basic sketch on top, read: *target* = skin; *reactive surface* = granulomatous barricade (formed from the connective tissue separating the derma from the local stressor); mild *stressor* = dilute croton oil; *pro-reactional factor* = STH; *anti-reactional factor* = cortisone. Dilute croton oil does not penetrate into the skin sufficiently to cause necrosis under normal conditions. *A*. The situation is unchanged because both STH and cortisone levels are proportionately raised. *B*. The granuloma is penetrated and the skin becomes necrotic because concentrated croton oil is used and, to resist it, a normal balance between pro- and anti-reactional factors does not suffice. *C*. Excessive amounts of STH are given, although the stressor is only dilute croton oil. The granuloma is far in excess of what would be required, and hence the great inflammatory response itself is the major manifestation of "disease." *D*. Cortisone is given in excess, therefore, virtually no inflammatory barricade develops, so that even dilute croton oil penetrates into the skin and causes necrosis.]

In essence, stress-therapy (treatment with adaptive hormones or nonspecific therapeutic agents) is a "*tactical therapy*," such as was practiced hitherto almost exclusively in surgery. There, it was always customary to use it whenever the causative pathogen could not be eliminated. For instance, when an inoperable neoplasm occludes the gut, the surgeon makes an artificial opening to by-pass the obstruction. This is no cure but it helps the patient to live with his disease in relative harmony. Thus, we accomplish by purely mechanical means what stress-therapy achieves through biochemical processes.

Stress-therapy is *not offensive;* it is not specifically directed against any one pathogen (as are antisera or chemotherapeutic agents).

Stress-therapy is *not symptomatic, nor strictly substitutional,* it does not act by merely eliminating any one specific symptom (*e.g.*, a headache with aspirin) or patching up a defect (*e.g.*, a loss of skin with a graft, a lack of vitamin with a curative amount of it).

Stress-therapy is rather *tactically defensive,* in that it adjusts active demarcation (barricading) and passive submission (topical death and spreading of pathogen) in a manner favorable to the organism as a whole.

Some agents are virtually "*unconditional pathogens*," in that their influence upon the tissues is so great that they cause disease almost irrespective of any conditioning or sensitizing circumstances. (*E.g.*, X rays, severe thermal or mechanical injuries and certain microorganisms to which everybody is susceptible.)

Most disease-producing agents, however, are to a greater or lesser extent, "*conditionally acting pathogens*": that is, they cause maladies only under special circumstances of sensitization. Their pathogenicity may depend upon genetic factors, the portal of entry through which they are introduced into the body, the previous weakening of resistance through malnutrition or cold, etc. Here, it is often impossible to decide which, among the many factors necessary for the production of disease, is the pathogen and which the conditioning factor, because the full *pathogenic situation* must be realized before the malady can become manifest.

Search for Further Unification

This interpretation was still not wholly satisfactory as a unifying concept of disease, primarily because it failed to encompass the *noninflammatory maladies*. With the characterization of the L-A-S it became evident, however, that local stress produces not only inflammation, but also degeneration, necrosis, hypertrophy, and hyperplasia. All of these changes can be regulated by pro- and anti-phlogistic hormones whose actions (as well as the corresponding effects of vasoconstrictor and vasodilator nerves) might therefore be viewed more properly as favoring or inhibiting *reaction to injury in general*. Hence, the concept must not necessarily be restricted to the inflammatory diseases.

The question remains, however, *whether the manifold specific effects of agents could also be integrated into this concept*. These appear to be qualitatively different both from each other and from the non-specific (stress) effects; hence, apparently, they do not lend themselves to a unified interpretation. If we could somehow also express specific actions in terms of stress, all disease manifestations would be reduced to a common denominator. They would result from different proportions of the aforementioned three fundamental pathogenic factors: (1) the stressor; (2) pro-reactional factors, and (3) anti-reactional factors.

Apart from the relative proportions between these three factors, the manifestations of maladies would then only depend upon *when, where, and how much*, this same triad develops. That is, upon: (1) *time* (the duration and possibly repetitive nature of stress); (2) *space* (the location of the target or targets); and (3) *intensity* (the degree of activity, at which any given proportion between the above mentioned three fundamental pathogenic factors could act).

During this year, we attempted to formulate the conceptual foundation for this view through the "*reacton*" theory. The latter was discussed at length in two special reports (Selye, 1953a; Selye and Horava, 1953) upon which the following outline is based. By way of an introduction, let us state at once, however, that we believe the common denominator between the manifold specific and nonspecific actions to be that they all are merely mixtures of the same kinds of elementary responses.

Although biologic reactions tend to give the impression of oneness, they actually represent mosaics of simple activation and/or inhibition (stress) in a great variety of pre-existent elementary, subcellular biologic units, the "reactons." Each of these is capable only of one kind of response, but by blending these elementary reactions in different combinations, qualitatively distinct aggregates result.

To illustrate this by an example, let us remember that one can create a virtually unlimited variety of melodies on the keyboard of a piano, by merely "activating" or "inhibiting" its chords, varying time, site and intensity of simple touch on pre-existent keys, each capable only of one kind of response. We hope to show that "reactons," not cells, are the elementary "keys" of living matter and that all the manifestations of normal and pathologic life depend only on when, where, and how much (or how many of) these biologic elements are stressed.

What is Apparently Distinct and in Need of Unification?

In thinking about the fundamental nature of any phenomenon, the human mind invariably tends to analyze it from two essentially distinct viewpoints. We want to know its *primary cause* and its *primary constituent element*.

For instance, if an intelligent member of a primitive tribe were first confronted with an automobile, he would ask himself, "Who made it?" (that is to say, what is its cause?)—but also, "What is it made of?" (that is, what are its constituent elements?).

This innate quest for the Primary is also quite evident during the period of mental awakening in every child. It manifests itself by what might be called the "serial why," which leads to the following type of conversational pattern: "Why is it dark at night?" "Because the sun sets." "Why does the sun set?" "Because the earth turns away from it," and so forth, until the hard-pressed adult succeeds in changing the topic.

Our craving to climb up along such question-ladders does not diminish with maturity; only our hopes of reaching the top rung fade away with age, for we come to realize that it is just as inherent in human nature to be blind for the Primary as it is to look for it. Yet, as soon as man understands that, for him, the ladder of comprehension has no end, he can find comfort in the realization that consequently, there also is no limit to his possible prog-

ress; no matter how advanced his wisdom, he always remains capable of yet another step forward.

Except for a few remarks in the chapter on teleologic thought, we shall have little to say about primary causes in medicine, but our experimental work has led us to a point where we feel that a better understanding of the primary elements of disease could be of great assistance in sketching a more unified picture of medicine. Before attempting any further unification, however, we must try to outline what strikes us as being especially in need of it.

What are those concepts of medicine whose distinctness is in question? For this lecture I have merely selected five pairs of twin concepts, because they are rather basic in biology and medicine and because, at first sight, they appear to defy any effort of being reduced to a common denominator.

(1) *Health and disease.* Textbooks usually define health as the absence of disease, and *vice versa.* The two conditions are alleged to be opposites of each other. Is this really so? Are they not rather different only in degree and in the position of vital phenomena within time-space?

Hemorrhage, inflation, loss of weight or a rise in temperature could be mentioned as typical manifestations of disease, in that they supposedly represent a deviation from the condition of health which we call the norm. However, menstruation is accompanied by hemorrhage and inflammation yet, the failure of its occurrence is what we would call a disease in a young adult woman. If she menstruated excessively or from any place other than the uterus (*e.g.*, in endometriosis), that would also be a disease, as would be menstrual bleeding at an abnormal time (*e.g.*, in precocious puberty).

A loss of weight, a variation in temperature can likewise be considered as pathologic only in relation to some norm of health, but even health and biologic normalcy are not synonymous. An individual born with an undeveloped toe or one with a disfiguring scar across his face is neither normal nor unhealthy. A defect is not a disease.

It may be argued that the examples given: homorrhage, inflammation, loss of weight, a rise in temperature are merely manifestations of disease and not disease itself. Perhaps we should distinguish between disease and the signs or symptoms of disease.

(2) *Disease itself and its signs or symptoms.* The dictionaries tell us that disease is "a definite morbid process having a characteristic train of symptoms. It may affect the whole body or any of its parts, and its etiology, pathology and prognosis may be known or unknown." On the other hand, a symptom is "any functional evidence of disease," while a sign is "any objective evidence of disease" (Dorland's Medical Dictionary).

This point of view, though quite generally accepted, is hardly satisfactory to the analytical mind. Is the high blood-pressure of essential hypertension a disease or a sign? If it is a disease, it is also its own sign and, if it is a sign, what is it a sign of? The same doubts arise in connection with nephritis or pneumonia. These conditions could be called diseases, while their consequences (*e.g.*, proteinuria and hypertension with nephritis, fever and dyspnea with pneumonia) might be regarded as their signs. Yet, if the same renal and pulmonary changes happen to develop in the course of say, scarlet fever, then they are no longer considered to be diseases in themselves, but signs of the latter malady.

Actually, we have a generally subsconscious but very practical reason which largely justifies this usage. When we speak of disease, we actually mean that there is no hope, in the light of available knowledge, of ascending any higher in the causality chain of the condition before us. When we speak of a sign, we mean that there is hope that we could climb at least one rung higher towards the understanding of the primary cause.

Thus, when we compare the concept of disease with that of the symptoms and signs, the difference does not appear to be essential and we are tempted to ask whether some unifying interpretation of these derangements could not help us to arrive at a more fundamental understanding of morbid phenomena. Of course, when it comes to signs and symptoms, the first preoccupation of the physician is the degree of their specificity, that is their reliability as indicators of any one disease.

(3) *Specific and non-specific phenomena.* The original meaning of the term specific is "that which characterizes or constitutes a species." For instance, we speak of the specific difference between the dog and the wolf.

The concept of specificity is of particular importance in stress-research, since we have defined biologic stress as a condition elicited by preponderantly non-specific agents, that is, agents

acting on many targets without selectivity. By contrast, a specific agent acts only on one or few targets and therefore, provokes selective effects. Thyrotrophic hormone has been mentioned as an example of a specific agent because few, if any other substances share its selective action upon the thyroid epithelium. Conversely, many of the changes elicited by pyrogens are rather non-specific, since they can be duplicated by numerous other agents. Yet, it would be difficult to mention any example of an absolutely specific or absolutely non-specific agent.

Here again, we gain the impression that the two partners of the conceptual pair, specific and non-specific, are only apparently different in quality, but actually distinct only in degree, that is in quantity.

(4) *Qualitative and quantitative differences.* This brings us to the very crucial problem of distinguishing between qualitative and quantitative differences in general. One would think that the actions of a mild and a potent pressor substance differ only in degree (quantity), while for instance, the diametrically opposed effects of a nervous excitant and a depressor drug differ in quality. However, this distinction appears to be self-evident, only because of the terminology used. Speaking about excitation and depression implies that we view these conditions from an intermediate level, which we recognize to be the "norm." If we begin from absolute lack of activity, we can construct an ascending scale, which rises from zero, through increasingly less pronounced degrees of "depression," to "excitation."

It is perhaps not wholly unjustified to ask whether qualitative and quantitative differences are not likewise merely a matter of degree and of the distribution-pattern of vital phenomena in time and space. One could imagine, for instance, that the impression of virtually any qualitative difference could be created by mere combinations and permutations of induced activity, in a limited number of biologic elements, each capable only of a single kind of response.

This brings us to the last and perhaps the most important twin concept, which we shall have to analyze before attempting to arrive at some unified interpretation of biologic phenomena, namely: the problem of the fundamental unit of life.

(5) *Units and Complexes.* The difficulty of drawing a sharp distinction between units and complexes is, of course, by no

means particular to biology and medicine. We encounter it, for instance, in chemistry, physics, algebra, and geometry. We speak of a unit of matter, or of energy; a number can be taken as a unit, and so can a certain element of structure or form. To mention a classic example, the atom has long been considered to be the unit of matter. However, in view of recent progress, particularly in physics and mathematics, this apparently indivisible elementary building block has been shattered, and from its fragments emerged the most universal theory of unification, which the human mind has yet been able to create. And still, the atom is a unit. It only is not the ultimate or fundamental unit. In biology, a species is a unit among living forms, and so is an individual within this species, an organ within this individual, a tissue within this organ, or a cell within this tissue. Traditionally, the cell has been considered to be the primary or fundamental unit of life and yet, it is still a highly complex aggregate.

In the following section, we shall explore to what extent the stress-concept could help us to breach the gaps between the aforementioned basic concepts of biology and medicine: health and disease, disease and its symptoms, specific and non-specific changes, qualitative and quantitative differences and, most fundamental of all, units and complexes.

I do not know—nor does it really matter—whether the basic arguments in support of my views have already been expressed by philosophers. Probably they have. Priority of discovery, in the sense in which it exists in the natural sciences, hardly has a true counterpart in the science of thought itself. Every pattern of argument, compatible with the structure of the human brain, has probably been formulated at some time, in some language or form, by some author, especially as regards such basic principles as those with which we shall have to deal. But there is a great difference between a thing that has been seen (or even described) and one that is known to those whom it concerns. The basic procedures of reasoning are equally applicable to all sciences, yet in order to use them for the creation of new knowledge and understanding in any one domain, they must first be translated into a language, understood and accepted by the workers in that particular field. To us, biologists and physicians, generalizations are acceptable only if they are demonstrably substantiated by many measurable individual data. We instinctively shy away

from philosophic arguments, because the mind works much more rapidly than the hand, and as soon as we risk to formulate some generalizations about our science, we realize that the laboratory cannot keep pace with imagination. Ours should really be an experimental philosophy, since it is only the teachings of objective observation that we can really assimilate. As its natural counterpart, such an experimental philosophy would create a theoretic medicine which, I believe, could become just as enlightening and practically useful to us physicians, as theoretic physics has been to our colleagues in the exact sciences.

To lay the foundations for this will necessarily be a time-taking endeavor, likely to require the concerted efforts of several generations. The following pages can be regarded only as a very tentative and hesitating attempt to draw a few lines for a preliminary sketch.

How Could the Stress-Concept Lead Us to a More Unified Interpretation of Medicine?

(1) *Stress and Adaptation.*—Our earlier work on the general adaptation syndrome (Selye, 1952) and particularly, the more recent observations on the local adaptation syndrome (Selye, 1953b), brought out clearly the essential difference between "specific resistance" and "crossed resistance." They convinced us of the necessity to distinguish between three fundamentally different types of adaptation:

(I) *Homotropic adaptation,* that is, a simple, progressive adaptive phenomenon, which can be accomplished by mere hyperplasia and hypertrophy of pre-existing cell-elements, without qualitative change. This response is elicited whenever a tissue is only required to increase its activity, as regards a function to which it is already adapted at the onset. Such pre-existent adaptation can be the result of phylogenetic and ontogenetic development (*e.g.*, a muscle cell forced to perform more than the usual amount of contractile activity), or of previous adaptive transformations (*e.g.*, a macrophage formed from undifferentiated mesenchyme under the influence of India ink, which is now forced to engulf additional amounts of the same substance). The purely morphologic aspects of this are sometimes designated as "prosoplasia."

(II) *Homoiotropic adaptation,* which is called forth whenever a tissue adapted to one function is called upon to perform a slightly different, but cognate activity (*e.g.*, a macrophage adjusted to the phagocytosis of kaolin particles, which is brought in contact with carbon particles).

(III) *Heterotropic adaptation,* in which a tissue, fully differentiated for one type of action, is forced to re-adjust itself completely to an entirely differ-

ent activity (*e.g.*, transformation of a muscle cell into a polynuclear phagocytozing giant-cell). The purely morphologic aspect of this is "metaplasia."

In general, it may be said that the more a cell differentiates for a certain function, the less is it capable of heterotropic adaptation. Usually, the latter requires more or less complete dedifferentiation to a rather embryonic cell-type, since fully differentiated cell-forms can rarely transform themselves directly into one another. Mitosis is the most effective way of regaining full, juvenile adaptability, by divesting the cell of all its differentiation. Interestingly, mitotic proliferation is also rarest in the most highly differentiated cell-types.

Above we discussed the three types of adaptative phenomena principally in connection with topical stress. It is evident, however, that this same classification is also applicable to systemic reactions, since these are essentially composed of multiple topical responses, integrated throughout the body by nervous and humoral means. It will be observed that from the simplest homotropic to the most complex heterotropic, these adjustments are effected by varying proportions of simple growth of pre-existent tissue on the one hand, and qualitative transformations into new kinds of tissue on the other.

These morphologic characteristics of adaptive phenomena have their equivalents in terms of biochemistry. Simple anabolism (the mere addition of more of the same material) suffices for homotropic adaptation, while more or less complete transformation of chemical constitution is usually necessary to effect heterotropic adjustments. Dedifferentiation appears to be a necessary intermediate not only for the morphologic but also for the chemical processes of "transadaptation."

The catabolism which occurs both in topical and in systemic shock is, in a sense, a "*chemical dedifferentiation.*" It is through the lysis of cellular material (histolytic phenomena in the L-A-S, loss of body and organ weight in the G-A-S-) that chemical substances are liberated, which can serve as building blocks for the neoformation of tissues better adapted to new requirements.

This section on stress and adaptation revealed certain aspects of unity in apparently diverse vital phenomena. The chief unifying principle, in the emerging impression of "oneness," is the basic reaction-type to stress, with its concomitant adaptive re-

sponses. Yet, we are still far from understanding what meanwhile remains a simple impression of some fundamental unifying law. As the picture stands at this point, perhaps its most disturbing feature is the difficulty of correlating the "pathologic" phenomena of transformative adaptation (*e.g.*, inflammation) with the "physiologic" type of simple evolution or progressive tissue-growth. The latter appears to be directed by the laws of heredity, without any manifest dependence upon stress.

(2) *Stress and Growth.* It is noteworthy that the so-called "adaptive hormones" are also important regulators of *systemic growth.* ACTH and the A–Cs (cortisone, hydrocortisone) are potent growth-inhibitors, while STH is so effective in the opposite sense that it has actually been called "the growth hormone." It is in agreement with expectations, therefore, that stress itself can affect the growth of the body as a whole. Systemic stress has a marked growth-inhibiting effect. At least in part, this is mediated through ACTH and the A–Cs.

But is there any link between stress (or adaptive hormones) and that selective *topical growth* of certain parts, which leads to qualitative changes in morphogenesis? Of course inflammation, one of the most salient features of topical stress, is accompanied by particularly pronounced local, proliferative growth phenomena, which are often homotropic (*e.g.*, simple proliferation of pre-existent fibroblasts). Other examples in point are, selective lympholysis caused by systemic stress or by antiphlogistic hormones and lymphatic hyperplasia after STH treatment.

Little is known, as yet, about the possibility of inducing regional growth in certain areas with the aid of topically applied STH, but it is undoubtedly possible to induce a marked selective growth-inhibition by the local application of hydrocortisone to certain predisposed regions. Thus, the development of one paw, one ear, the snout or a specifically sensitized skin region can be greatly inhibited by topical treatment with hydrocortisone (Selye and Horava, 1952, 1953).

In many of these instances, the topical effects of hormones, either upon inflammation or upon growth, are manifest only under certain conditions. For instance, systemic stress or local vasoconstriction sensitizes to the topical effects of catabolic (antiphlogistic) hormones, while freedom from systemic stress or local hyperemia enhances the topical actions of anabolic (pro-

phlogistic) substances, such as STH. It has become a major branch of stress-research to explore the mechanism through which these "conditioning factors" alter regional tissue-reactivity to hormones [for reviews see "STRESS," "First Annual Report on Stress," "Second Annual Report on Stress," "Third Annual Report on Stress" (Selye, 1950, 1951; Selye and Horava, 1952, 1953)].

It is also possible that, during topical stress, certain cells may develop a special affinity for growth-regulating adaptive hormones, although the available bio-assay methods have not yet permitted us to establish this with certainty. All these factors could endow certain tissue-regions with a selective sensitivity for one or the other type of adaptive hormone and thus, permit the participation of stress in localized tissue-growth phenomena.

Perhaps the most important topical stimulant of growth is activity. A muscle cell, forced to perform much contractile work, or a glandular cell, stimulated to excessive secretory activity, will undergo hypertrophy. Could the "stress" of local hyperactivity itself act as a "conditioning factor" for growth-regulating adaptive hormones? By calling this "stress" we may appear to obscure the limits between specific and nonspecific actions, a distinction which is the very basis of our stress theory.

(3) *Stress and Specificity*. If stress is defined as a nonspecific action-reaction phenomenon, then adaptive tissue-growth, elicited by highly specific stimuli, cannot be so designated. Thus, the selective hypertrophy of a muscle caused by frequent stimulation with its specific physiologic excitant, the motor-nerve impulse, is certainly not a stress-reaction in the usual sense. It is equally evident, on the other hand, that such specific muscular activity, if excessive, can cause local damage to the muscle itself, as well as systemic stress, with the accompanying manifestations of the G-A-S, for instance, an ACTH discharge, lymphatic involution or gastro-intestinal ulcers. What then, is the relationship between the specific and the nonspecific in such responses?

This leads us to the frequently discussed, but still quite cryptic question of the "*first mediator*" of stress-reactions.

A localized tissue-injury (*e.g.*, one caused by a burn or croton oil), if sufficiently severe, produces not only topical manifesta-

tions of the L-A-S, but also a mobilization of systemic defense through the G-A-S. Yet, we still do not know through what mediators the impulse is carried from the site of injury to distant parts of the body, for instance, to the pituitary-adrenal axis. The integrity of the innervation in the injured area is not essential, since damage to a completely denervated limb can still elicit a G-A-S. Therefore, humoral stimuli alone appear to be adequate for the generalization of topical stress-responses, but of course, nervous stimulation can also act as a systemic stressor.

The humoral transmittor substance, or substances, in question have been described, provisionally, as the "first mediator(s)." Various investigators considered the possibility that locally produced nonspecific products of tissue-catabolism, polypeptides, histamine, histamine-like compounds, adrenergic or cholinergic substances might be the "first mediator." It has been demonstrated beyond doubt that a G-A-S can be produced with any one of these compounds, but all efforts have failed to prove that one of them is the only "first mediator" of all stress-reactions. Indeed, it is even possible that the mediation is not due to the presence of humoral substances, but rather to the lack of some vitally important blood-constituent, which might be avidly used up by damaged tissue, to the detriment of the organism as a whole.

In the genesis of inflammation, "mediators" have also been postulated. Here again, polypeptides (*e.g.*, leukotaxine), histamine, histamine-like substances, and many other alleged regulators of the inflammatory process were the subjects of much study. The fact that a stimulus for inflammation can be thus transmitted by humoral means is definitely established. To take but one example, the exudate which accumulates in a granuloma pouch,[1] produces inflammation in connective tissue wherever it is injected. Indeed, we found it possible to first produce exudate (with the minimum effective dose of croton oil, in a granuloma pouch) and then to transmit inflammation with it through several animal passages. Fluid formation in response to injected exudate remained strong, even after three or four trans-

[1] The granuloma pouch is made by injecting a given amount of air subcutaneously. This is followed by injection of an irritant (*e.g.*, croton oil) into the regular ellipsoid chamber so formed. The irritant transforms the connective-tissue lining into a granulomatous barrier, while the air is gradually replaced by fluid exudate (Selye, 1953b).

fers. By that time the original irritant (croton oil) was so much diluted that it could no longer be responsible for the inflammatory reaction. Hence some humoral transmittor must be postulated, but the nature of this "phlogistic mediator" remains unknown.

The question now arises whether the "first mediator" of the G-A-S (the endogenous stimulus which transmits the stress-message from the injured region to the whole organism, and particularly to the pituitary) could be the same as the "phlogistic mediator of the L-A-S (the endogenous stimulant of inflammation). Injection of exudate is highly effective in producing a systemic alarm reaction with pronounced ACTH discharge. Perhaps the products of tissue-injury and tissue-activity, if formed in sufficient concentration, are responsible not only for topical defense (L-A-S with inflammation) but, if also absorbed in sufficient concentration, they could even alert the whole organism (by eliciting the G-A-S). None of the data available at present disprove the possibility of such a close relationship between local and systemic stress-responses through the same system of mediation.

With this in mind we might now reconsider the somewhat confusing point raised at the end of the preceding section on "Stress and Growth." Is there any *relationship between non-specific stress and specific adaptive growth* (*e.g.*, of a muscle specifically stimulated by motor impulses)?

In this connection, let us recall that intense over-exertion of a muscle can produce a true myositis. Inded, if such a muscle is subsequently exposed to topical irritants, it reveals a marked change in non-specific local reactivity (Barany, 1933) phenomena which we would interpret today as "crossed resistance" or "crossed sensitization." Thus, specific stimulation of a target to perform its physiologic work can act as a topical phlogistic (topical stressor) stimulus, at least at certain dose levels. Of course such specific physiologic stimuli can also cause work hypertrophy. Thereby work predisposes the target to the homotropic (simple adaptive-growth stimulating) actions of anabolic hormones, for instance, of STH.

Another relevant point is the *relationship between specificity and quality of response.* Non-specific agents have been defined as stimuli "which affect many targets and are devoid of the ability

to act selectively upon any one" ("AROS-1952"). By definition the reverse is true of specific agents. Similarly, a non-specific change is one which can be elicited by many agents, while a specific change can only be produced by one, or at least, a very limited number. The two appear to be quite unrelated and different in quality. Indeed, one has come to consider them as opposites.

The follicule stimulating hormone is an example of a highly specific agent, since few, if any other stimuli share its power to exert a direct, selective stimulating effect upon the granulosa cells. Conversely, the production of an alarm reaction by exposure to cold is quite non-specific, because numerous other stressors (trauma, infections, nervous stimuli) act this way.

Yet, even this fundamental difference between the specific and the non-specific is perhaps more apparent than real. It has been shown for a great many reactive elements ("receptors" or "targets") that they can respond to irritation only in one manner. Their reaction-type is conditioned by their own structure, not by the stimulus which activates them, although they may be more sensitive to some agents than to others. Whether stimulated by heat, mechanical injury or electricity, a muscle-fiber reacts with contraction, an optic nerve-fiber with the sensation of light, a glandular cell with secretion, and so forth. Could it be that the apparent multiplicity of "specific" reactions is merely due to combinations and permutations of such single reaction-types, of which the various biologic elements of the body are capable? Should this be the case, then all the manifestations of life, from the regional to the systemic, from the entirely non-specific to the most highly specific, could be brought down to a common denominator. They would merely represent various groupings of simple, qualitatively unidirectional, responses in the diverse biologic units (organs, cells, cell-parts) of the body.

Thus, the fundamental uniformity of any vital response—growth and adaptation, reaction to topical or to systemic stressors—begins to emerge in a more definite form. One great problem which remains is the precise nature of the interrelations between the specific and the non-specific responses. We believe that the understanding of this has been particularly handicapped by the general acceptance of the theory, which assumes that the cell is the ultimate unit of living matter. *Even a single cell can respond in qualitatively different ways. It would be difficult to understand*

this without assuming the presence within the cell of smaller units, which are still relatively independent of each other in their reactivity.

(4) *The Functional Unit of Life.* In 1667, through his primitive microscope, Robert Hooke, saw minute compartments in living plant tissues. These appeared to be empty spaces, separated by walls; he therefore called them "cells." Subsequently, it became evident that these cells are actually filled with what appears to be living matter, they contain a nucleus and cytoplasm. Yet, it was not until more than two centuries later that Schleiden and Schwann (1839) were struck by the fact that all living matter (including both plants and animals) is made up virtually of cells alone. This led them to propose the first great truly unifying scientific concept in biology, the so-called "cell-theory." In brief, they assumed that the cell is the fundamental unit of all that is alive, somewhat as, for some time, the atom was considered to be the fundamental indivisible building block of matter.

The heuristic value of this concept for biology and medicine was as great as that of the atomic theory for chemistry and physics. Virchow's "cellular pathology" and many of our most fundamental biologic concepts were based on it. It is the essence of science to explore causal and topographic relations between units. No wonder that the recognition of a visible unit of life helped the progress of biologic science!

However, subsequent research revealed many facts incompatible with the cell-theory of life. Certain slime moulds, for instance, grow to considerable size and, although they contain nuclei, they show no sign of a subdivision into cells. Conversely, erythrocytes contain no nucleus. The configuration of viruses is even more remote from the cellular structure, still they do appear to be alive. And why should we consider inter-cellular substances as inanimate?

Despite these and many other facts, patently incompatible with the cell-theory, the latter was so useful that it became too deeply ingrained in the minds of biologists to be displaced by any other concept; findings which did not fit into it were merely discarded as unimportant exceptions to the rule. After all, even the atomic theory, which postulated the inconvertibility of elements into each other, had its exceptions.

However, as we know now, the atom is divisible and does not represent the fundamental unit of matter. Consequently, it has been possible to effect qualitative changes in elements—although they consisted only of one kind of atom—by rearrangement of their true fundamental units. Yet, for a long time work along these lines was actually handicapped by the otherwise so fruitful, atomic theory of matter. Could these same considerations not also apply to the cellular theory of life?

We already had occasion to mention that certain targets or receptors of biologic stimuli are of subcellular dimensions. It is inconceivable that the cell should be the fundamental unit of living organisms since, in a single cell, various portions can perform diverse vital functions independently and simultaneously (*e.g.*, phagocytosis, secretion, perception, locomotion, digestion). An agent can undoubtedly influence one part of the cell (*e.g.*, an organelle), or one biochemical unit (*e.g.*, an enzyme system), selectively. These elementary biologic targets—we called them (Selye and Horava, 1953) "reactons," in analogy with other elementary units (such as the nephron or neuron)—are perhaps also "alive." At least, in the absence of evidence to the contrary, we must consider this possibility, since they exhibit the features regarded as characteristic of life, just as the cell. Among other things, such reactons can grow and reproduce their own kind. They also have a great tendency to maintain their characteristic individuality, despite changes in the *milieu*, that is, they are highly adaptable.

Let us point out clearly that nothing in our observations would justify the conclusion that these elementary targets are necessarily somatic structures, that is, matter. It is equally possible that the reaction is only a focus of interrelations, a functional plan or pattern, which governs the organization of matter. A plan possesses, to an exquisite degree, such accepted characteristics of life, as the ability of growing, reproducing its own kind and adapting itself to changing requirements. The living is undoubtedly matter, but life is only one of its characteristics.

The great strength of the cell-theory is that one can see the limits of these "building blocks" and demonstrate that, virtually all living matter is made up of them. However, this only shows that the cell is *a morphologic unit*, but is it the fundamental *primary unit?* The organs are units in the body, the

tissues are units in the organs, the cells are units in the tissues, but why must we stop at this particular level in our analysis of living matter? The possibility of demonstrating clear borders by optic microscopy is hardly an adequate reason.

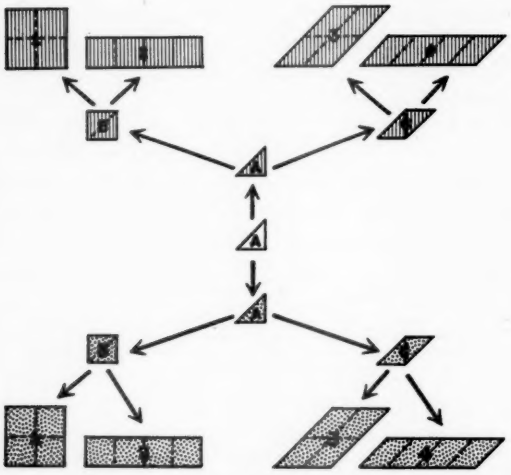


FIG. III. *Illustration of the view that "a specific element is the smallest part still specially fashioned for a certain composite structure." A.* The central blank "A" is a common element of "A" hatched and "A" stippled. The latter differ from the parent structure in shading, not in form. A whole series of structures can be created, with any type of shading, by joining the element "A," and its increasingly more complex aggregates, in different ways. Thus in either series shown here, "A" is a specific element of "B," as well as of "C." "B" is a specific element of "1" and "2," but not of "3" and "4," since the latter could not be constructed from it. Let us emphasize particularly, however, that "A" is not a *specific* element of any among the structures "1," "2," "3," or "4," but only of their precursors "B" and "C" respectively, since it is only up to this level that the shape of the small triangle (dotted line) is still distinctly implied by the outlines of the whole. In the same sense, the specific element of a species is the individual (not the cell), that of a tissue is the cell (not the reaction) and the smallest, but still specific element of living matter is the reaction (not the atom).

Where then, should we draw the line? Living, just as inanimate substance, consists of chemical elements, but these cannot be regarded as alive in themselves. They are units of matter, but not yet "live matter." Only certain combinations of the elements engender the characteristics of life. Then, why not recognize as the fundamental, specific elements of life, those

smallest, organized structures, which still exhibit selective reactivity to biologic stimuli and manifest the generally accepted criteria of life?

Let us see now whether this theory of the reactons could help us to understand phenomena which the cell-theory does not explain. The theory of the reactons postulates that:

(I) *The fundamental specific elements of life are of subcellular dimensions;* indeed they may not have visible limits, they may merely be focal points of interactions between the constituents in living matter. These "reactons" are defined as the smallest target, capable of selective biologic reactivity.

(II) *Each reacton can give only one kind of response.* The nature of this response depends upon the inherent structure of the reacton itself. Hence, at the level of these ultimate units, reaction-patterns cannot yet be separated into the specific and the non-specific. In other words, the concepts of quality and specificity of response have no meaning here.

(III) *Specificity of action depends upon the degree of selective affinity,* which an agent exhibits for certain reactions.

(IV) *Specificity of response depends upon the degree of freedom with which certain reactons can be activated* independently of others.

(V) *Intensity of response depends upon the number of reactons activated.* It is still to be determined whether the degree of activation has any importance at this fundamental level, or whether reactons are subject only to the "triggering" type of yes-or-no response, which necessarily leads to the complete discharge of the accumulated action-potential.

(VI) *Homotropic adaptation depends upon further work hypertrophy (and/or hyperplasia) of certain previously developed reactons.*

(VII) *Heterotropic "transadaptation" depends upon work hypertrophy (and/or hyperplasia) of certain undeveloped reactons at the expense of inactivity atrophy in others, which were previously developed.* It is this type of response which we have been accustomed to regard as a "qualitative" change. The schematic drawings on pages .. and .. might help to illustrate these thoughts by a simple, mechanical analogy.

At first sight, it may be difficult to comprehend how simple quantitative responses, in a limited number of reactons, might give the virtually unlimited number of, qualitatively distinct, reaction-patterns of which living matter is capable. Yet, it must be remembered that this is not without precedent in biology.

For instance, according to the Young-Helmholtz theory of vision, there are only three fundamental color sensations, red, green, and violet. By suitable combinations of these, all other colors can be formed. To explain this, it was assumed that there exist three kinds of nerve-elements in the retina, each of which is specifically responsive to the stimulus of waves of a certain

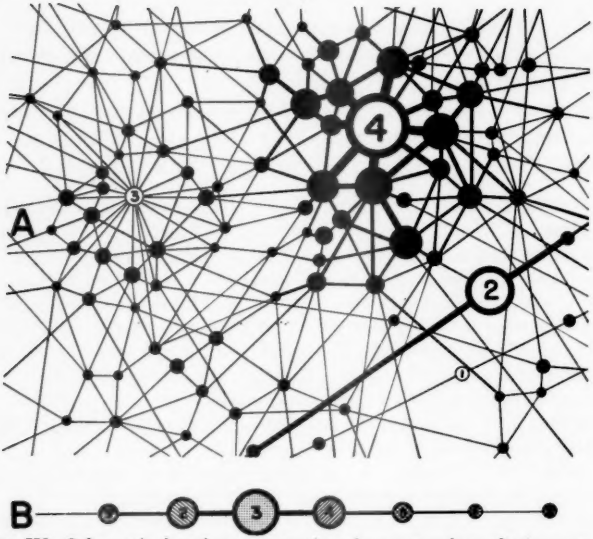


FIG. IV. *Schematic drawings illustrating the reacton hypothesis, as applied to the interpretation of specific and non-specific actions.* In all these drawings, the individual reactons are schematically represented by round bodies, and the connections between them (interactions) by straight lines. This is similar to the customary representation of atoms and valences, respectively, in chemical formulae. Although, actually, reactons are arranged three-dimensionally in living matter, they are shown here, in a single plane, for simplicity's sake. Four reactons, representatives of special conditions, are numbered.

Left. A. A field, containing numerous reactons (dots), in various stages of development ("surface view"). They are associated with each other by interactions of varying importance (straight lines). Thus, one might visualize them as the focal knots in a complex network of more or less obligatory or rigid interactions. Evidently, pressure exerted upon any focus will displace others as well, the extent to which local tension can spread will depend upon the number and strength of the connections between the directly affected focus and the rest of the system.

(1) Undeveloped reacton, directly connected only with two others.
(2) Highly developed reacton, directly connected only with two others.
(3) Undeveloped reacton, directly connected with many others.
(4) Highly developed reacton, connected with many others.

This illustrates that stimulation at a point, such as "1", will have a selective, specific effect (illustrated by "2"), while stimulation at a point, such as "3", will have a very generalized, rather non-specific effect, even though the agent acted at one point only (illustrated by "4").

B. Resting reactons. Sample of seven reactons, not exposed to any agent ("profile view"). One (No. 3) is most developed and the prominence of the others diminishes as we approach the periphery of the former's activity range. This is intended to be a graphic expression of a situation in which (due to genetic factors or previous activity) even at rest, one type of reacton is most developed, while the others have evolved only in proportion to the importance of their interactions with the former. (*E.g.*, in a developed muscle, the reactons directly and more or less indirectly related to contractility.)

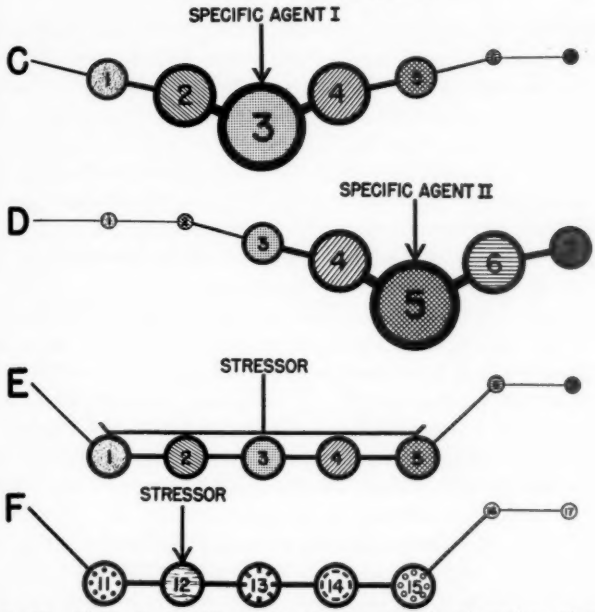


FIG. IV. *Right. C. Specific effect with homotropic adaptation.* "Specific agent I" acts upon the system represented in "B," stimulating it to perform the function for which it has already been specialized. This leads to "simple work-hypertrophy" with a proportionate development of all the pertinent reactons and of their interactions. (*E.g.*, in the above-mentioned example of the muscle, the repeated specific stimulation, through its motor-nerve.)

D. Specific effect with heterotropic adaptation. "Specific agent II" calls upon the system represented in "B" to perform a function qualitatively different from that for which it had been specialized. Now, the chief emphasis is upon a previously not fully developed reacton (No. 5). This leads to a shift with varying degress of "inactivity atrophy" in previously developed reactons (near the left end of the chain) and a corresponding "qualitative change" in structure and function. (*E.g.*, selective exposure of a single muscle group to heat, with resulting dedifferentiation of the myocytes into polynuclear giant-cells, suitable for the removal of damaged muscle-cell debris.)

E. Stress due to non-specific action. Here, the nature of the stressor is such that it affects all reactons and their interactions. The corresponding "dedifferentiation" is illustrated by a hypertrophy of the previously underdeveloped, at the expense of involution of the previously most developed, reactons. (*E.g.*, exposure of a large tissue-area to heat, which directly affects all its cells.)

F. Stress due to non-specific reacton. This also affects all reactons (and their bonds) in this field, although the stressor acts selectively on one (No. 12) alone. The reason for the generalization of the stress effect is the intense dependence of the units (Nos. 11–15) upon each other, in this particular field. Thus the response of one (No. 12) immediately produces marked repercussions in all others within the system. (*E.g.*, overstimulation of the adrenal nerves, whose direct effect is highly specific, but the resulting adrenaline-discharge produces a widespread stress-response.)

frequency, corresponding to one color. If the nerve-elements corresponding to red and green were simultaneously set in action, the resulting sensation would be orange or yellow; if mainly the green and violet, the sensation would be blue or indigo, etc. Actually, no such nerve-fibers or elements are known, but the theory is equally valid if the stimuli affect three photo-chemical substance-units. The innumerable melodies which can be derived from the simple keyboard of a piano (although each key can produce only one tone) have already been mentioned as another suitable analogy.

It is not too difficult to imagine that the many reaction-patterns of which any cell is capable could thus be "synthetized" by simple yes-or-no responses of its constituent reactons. In all these instances, the quality of the true fundamental element (as the pure color or pure tone) is invariable. Differences in kind cannot occur at the level of the elements; the impression of a qualitative change is created by blending the responses of the unchanging fundamental units.

The reacton hypothesis suggests experiments to test the feasibility of an *analysis and synthesis of cellular disease.*

If the concept is valid it should be possible to identify, by their characteristic actions upon the cell, common elements of activity ("actons") in various complex pathogens (microbes, drugs, rays, etc.) each affecting one type of reacton preferentially. [In chemistry, this would be comparable to the acidity (or the oxidizing power) of various molecules, which affect cognate receptive groups of various materials in essentially the same manner, and to a large extent, irrespectively of the other characteristics of the acid (or the oxidizing) molecule.]

Conversely, it should be possible to synthesize cellular disease by the application—in suitable order and intensity—of several agents containing the requisite combination of elementary pathogens (actons), even if none of these agents be endowed in itself with the whole desired pathogenic combination. [To take another analogy from chemistry, this would correspond to the synthesis of NaCl from NaOH and HCl or from other salts of Na and Cl, none of which exhibits the characteristics of table salt.]

The greatest weakness of the reacton hypothesis is that, at present, we possess no criteria which would permit us to ascribe

any elementary change within the cell (*e.g.*, the accumulation of certain granules) to a single reacton. Since the latter can be defined only as the smallest unit capable of selective reactivity, it is always possible that further research will reveal further subdivisions within the observed objective indicator (*e.g.*, the granule). However, this difficulty of spacially delimiting the individual reactons does not detract from the immediate practical applicability of the hypothesis, in the aforementioned sense. Even if now we cannot yet distinguish individual reactons from groups of reactons, the concept opens to experimental analysis that range of units between the cell and the chemical element.

Two analogies will help to illustrate our thought. It is of heuristic value to recognize that the color of an organ is not due to diffuse staining but to the selective accumulation of pigment granules in some of its cells. Observations made on this basis are not invalidated if it turns out subsequently that each granule contains several kinds of pigments. It was also of heuristic value to recognize that separate genes and not vague diffuse characteristics of living matter transmit inheritable characteristics much before the nature of the genes could be clearly delimited.

Apologia of Teleologic Thought in Biology and Medicine

It is evident that our whole unifying concept is based upon teleologic thought, that is the principle of purposeful causality. It is difficult to understand why, among representatives of the exact sciences, and even among biologists and physicians, there is so much resistance to the use of teleologic arguments. Still we must admit that many of the most outstanding investigators of our time believe that one can—and indeed should—merely register scientific observations, refraining from all considerations of causality. I cannot follow such arguments. To my mind the sensation of causality is inherent in the structure of the human brain. Understanding itself is but the feeling of having securely attached a thing to our treasury of known facts, by solid bonds of obligatory sequences.

Knowledge includes purely descriptive information (the answer to: "What is it like?"), but we can "understand" only the cause of a thing (the answer to: "What brings it about?"). In other words, we can know a thing by its characteristics (*e.g.*,

a house) or by the instinct of self-evidence (*e.g.*, $6 \times 6 = 36$), but we can understand it only in terms of its cause.[2]

This could be illustrated by the following diagram:

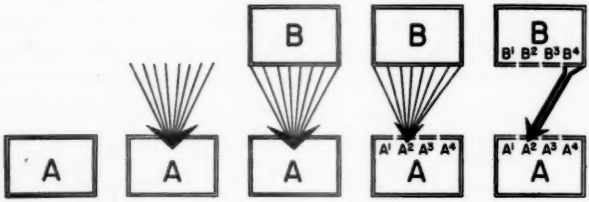


FIG. V. *Diagram illustrating the usual sequence in problematology as we attempt to approximate the understanding of primary causes and primary elements in experimental medicine.* (Read from left to right)—*1.* We discover a biologic target (*e.g.*, the adrenal). This gives us knowledge, but not understanding. *2.* We notice that the target can vary, it is capable of being changed by agents of meanwhile unidentified nature and origin (*e.g.*, adrenal enlargement). This gives us more knowledge, but still no real understanding. We ask ourselves "What is the cause of this change?" *3.* We find that the cause is "B" (*e.g.*, the hypophysis). This gives us the impression that we understand the cause of the change. *4.* However, we soon find that the apparent change of the whole is actually due to an alteration of only one of its elements "A^2" (*e.g.*, the adrenal cortex). Actually "B" acts on "A^2." *5.* Then we learn that not the whole of "B" but only "B^4" is the cause of the change (*e.g.*, ACTH). Thus arises the sensation of an increasingly better understanding. It is not a coincidence that, as understanding deepens, the sequence of causality becomes increasingly more obligatory. "B" does not always act on "A" but only if it can reach "A^2." The response is still more predictable if "B^4" can be applied in abundance, since actually no other element of "B" is effective. The same argument applies to primary causes, when we begin to ask "What acts on 'B'?"

But teleologic thought implies more than mere causality. It suggests an aim, that is *purposeful causation.* It is really because of this aspect that so many biologists refuse to accept it. As applied to our topic, the problem is rather clearly exemplified for instance, in Schwartz's (1953) recent monograph on inflammation. He says:

"One should think that the exalted Law of Nature—to vary a famous saying of Anatole France—recognizes no difference between microbes and man. One cannot consider inflammation as a specific 'cleansing' measure, that is one for the conservation of tissues, any more than one can envisage malignant neo-

[2] In conversational English, the two terms are somewhat loosely used. For instance, we say that we "understand" a foreign word, but what we actually mean is that we know its meaning. This is really mere recognition by characteristics, and essentially different from true causal understanding.

plasia as having the 'purpose' or 'duty' to destroy organs: both processes are—just as all manifestations of Nature—*in themselves* aimless and purposeless phenomena."

We could certainly not avoid dealing with this problem in our discussion on stress. All our factual observations were arrived at through experiments planned on the assumption that stress-responses are purposeful homeostrtic reactions. We must realize that in both of the above mentioned examples, there are two "teleologic centers"; their interests are opposed, but within each of them purposeful activity "for its own good" is clearly recognizable. On the one hand is the interest of the patient, on the other that of the microbe or the neoplastic growth. Indeed, the very essence of neoplasia is the setting up of a center whose own interests are largely opposed to those of its host.

Of course centers of homeostatis can exist within other such centers and, as soon as that is so, their interests will not be identical in every respect. To take an example, a citizen shares many interests with all his countrymen but, in ascending order, he shares even more with all those who live in the same city, or the same house. Not only bacteria and tumors, but even our normal tissues constantly compete with each other (*e.g.*, for space or blood); hence every cell, indeed every reacton within the body, represents a teleologic center, whose purposeful reactions must be analyzed in relation to all other centers. Indeed the terms "teleology" and "purpose" can be meaningful only in relation to an identifiable center. "It is advantageous" means nothing unless we say for whom.

Curiously, the formation of what we might call "teleologic centers" within the Universe appears to be one of the great laws of Nature. The stability of the most diverse structures appears to increase up to a certain optimum size or degree of complexity. The acquisition of this stability strikes us as an aim since everything tends towards it. After they reached their optimal complexity, all structures tend to become unstable and "die" by falling apart, so that their elements may initiate another similar cycle. In its product, it is rare to find exactly the same elements, arranged in the same manner as in the parent structure, because precisely the same conditions are not likely to prevail twice in succession. This leads to a constant slow evolution towards more and more stable structures.

We see this in the case of small drops of mercury which, upon contact, tend to aggregate into a larger, more stable drop. This goes on until a certain optimal dimension is reached, then the drop becomes unstable because of its size, and falls apart. We see this when a crystal develops, or an icicle, or an amoeba, or a human being. They only grow and fall apart in different ways. There can be simple shattering or disintegration (crystal, icicle), which makes all parts immediately available for totally different constructions. There can be direct and complete division into smaller structures, similar to the parent body (amoeba). In this case, there is no "corpse" from which to construct something totally new. And finally, an aggregate can fall apart by a combination of the two mechanisms (man) where a large part of the body disintegrates totally, yielding up its units (the "corpse") for wholly different constructions, while another part, the germ cell, is preserved for reproducing similar offspring.

Everything that persists for any length of time—that is, everything with any degree of stability—acts as a teleologic center in this sense. For this persistent activity it does not need the continued directing influence of an intelligent external influence with a purpose. Even a man-made object, once finished—an automobile for instance—will give us the impression of continually looking out for its own interests; it will meet hostile influences with "intelligently planned defense reactions." When driven along the road, it will cool its own motor, to prevent it from becoming damaged by heat. It will protect its body against shock with the aid of the springs and, indeed nowadays, if it has self-sealing tires, it will even be capable of true "wound-healing" if punctured.

Of course someone has to make the automobile, and someone has to make the maker of the automobile, and this leads us to the point where our teleologically constructed brain can no longer follow, for we cannot understand anything without a cause.

We sense a Creator, mainly because we and our surroundings strike us as being "complex" and, during the short life-span of man, he sees no really complex structure being built up by chance, without the catalytic influence of a Maker. Why doubt, however, that in the span of ages, the organizing effect of a

centralizing teleology could eventually build up awe-inspiring complexities, such as a planet, a tree or even ourselves?

Having been built and being capable of building are the most imminent characteristics of our every part. Hence we see everything through an atmosphere of building which tinges all our perceptions, just as a red crystal-ball, were it "alive" and capable of perceptions, would probably see everything as red, in and outside it. Teleologic thought does not necessarily have to lean upon a purposeful Creator, nor should it do so, even on religious grounds, since faith should not need the support of understanding. What we must clearly realize in biology is that teleologic analysis is applicable to any unit of creation, even after is is made.

Science cannot and should not attempt to embrace the purpose of the original Creator, but it can and constantly must examine teleologic motives in the objects of creation, since only this can lead to understanding, as opposed to a mere accumulation of unintelligible facts.

General Summary and Conclusions

In the first part of this lecture, we gave a brief synopsis of those experimental observations upon which our stress concept is based.

It was demonstrated that the current theories of the general and local adaptation syndromes are compatible with all these facts. They suggest certain avenues for further research, particularly as regards the mechanism of the so-called "non-specific therapy."

For the purely practical purpose of rationally directing current laboratory and clinical research on stress, there is no point in entering further into the theory of this topic. A more profound theoretic analysis would be required, however, to impart greater precision to our thoughts concerning the essence of such fundamental and yet so vaguely formulated concepts in biology as: "Specificity" and "quality" of action, "adaptation," "differentiation," "stress" and, particularly a "unit" of living matter.

Vagueness of terms and concepts passes unnoticed, as long as there are not sufficient objective observations to which these should refer. Indeed, it would be a futile dialectic exercise to aim at conceptual or terminologic precision in reference to a field

which has not yet been subjected to that degree of laboratory analysis which makes such theoretic precision possible, or even necessary. Yet, the theory must always be a little ahead of the facts, otherwise it cannot lead us to them.

In the early days of research on the adaptation syndrome, we managed well with the concept of biologic "stress." It was only later that we became handicapped by the realization that the term (and concept) failed to distinguish between the agent (cause) and the condition (effect). Only then could we separate the "stress" from the "stressor," indeed, only then did such a distinction become useful.

We believe that now objective laboratory research has progressed sufficiently far to attempt a more precise formulation of the concepts mentioned above, because their vagueness, in our mind, proved to be a constant stumbling block in the interpretation of stress-reactions. This is our excuse for the speculations which constitute the second part of this address. This does not lend itself to a brief synopsis, but it might be profitable to recapitulate, here, a few of the leading thoughts which have guided our theoretic analysis.

(1) The observation that *stress* can be accurately assessed by its objective criteria, permitted us to define it as a nonspecific adaptive syndrome.

(2) By studying the chronologic evolution of systemic stress, we arrived at the concept of the "*general adaptation syndrome*" (G-A-S).

(3) An enquiry into the effect of stress applied to circumscribed tissue regions, revealed the existence of a closely related, but topographically more limited "*local adaptation syndrome*" (L-A-S).

(4) This naturally led us to explore the behavior of individual cells under stress. It became evident that, while undergoing adaptation, even a *single cell can respond with many qualitatively different biologic reaction-forms*, both specific and nonspecific.

All these studies, on the general and local adaptation syndromes, sharply highlighted the difference between: *homotropic adaptation* (simple quantitative progression along phylogenetically and ontogenetically established lines) and *heterotropic*

adaptation (dedifferentiation to secure building blocks for subsequent qualitative reconstruction).

Since even a single cell proved capable of heterotropic adaptation by rearrangement of its biologic elements, it could not be itself the fundamental unit of living matter.

(5) This led us to the hypothesis of the "*reactons*," which postulates that subcellular units can still exhibit the generally accepted characteristics of life.

In the course of evolution, living matter has become increasingly more complex.

Life is difficult to define, as there does not appear to be any sharp line of demarcation between it and the inanimate. It is perhaps best defined by the degree to which it has developed certain characteristics, particularly those of recreating its own kind (growth, reproduction) out of less highly organized materials, and of maintaining its structure, tenaciously, despite any environmental changes that would tend to destroy it (adaptation). All these characteristics are recognizable even in the most elementary biologic targets, the reactons.

Simple chemical compounds never exhibit these qualities to any great degree. Hence, we do not regard them as living. The now most generally accepted theory postulates that the cell is the smallest unit that can be considered to be alive, but many facts are incompatible with this view. Neither microbes nor viruses exhibit the characteristic features of cells, yet they appear to be alive in the classical sense of this word. Furthermore, a single cell can respond in qualitatively different ways to stimuli (*e.g.*, phagocytosis, locomotion, secretion), and even its constituent parts exhibit the powers of reproducing their own kind (*e.g.*, growth of organelles) and of maintaining their structure, despite external forces which tend to destroy it (*e.g.*, reconstitution of secretion granules after discharge). Real units may be large or small, numerous or few (quantitative difference), but by definition, they cannot be composed of diverse elements (qualitative difference).

(6) There is no evidence that new reactons can be created from any source other than preformed reactons of the same kind. The old adage "Omnis cellula e cellula ejusdem generis" did not really hold true for the cell (*e.g.*, metaplasia, neoplasia), but

it might perhaps be reformulated more correctly by saying: "*Omne reacton e reactone ejusdem generis.*"

(7) In the light of this hypothesis, we attempted to formulate some of the fundamental concepts in biology, as follows:

growth = multiplication or enlargement of reactons

specificity = selective responsiveness of certain kinds of reactons

homotropic adaptation = further activation and growth of previously developed reactons

heterotropic adaptation = activation and growth of dormant reactons, with relative regression of those which were previously most prominent.

(8) As we see it now, the most fundamental task will be to find strictly objective means, with which to test the validity of our principal deduction, namely that: "*all vital phenomena depend merely upon quantitative variations in the activation of pre-existent elementary targets.*"

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THE EFFECT OF CHOLESTEROL-FREE DIET ON SERUM CHOLESTEROL IN DOGS*

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In our studies on altered thyroid function and cholesterol in dogs (Futch, *et al.*, 1949; Tsai, *et al.*, 1953), it was necessary to eliminate the influence of exogenous cholesterol by feeding a cholesterol-free diet. This opportunity was utilized to observe the effects of a cholesterol-free diet on serum cholesterol level in dogs inasmuch as such data on dogs were not found in the literature.

Material and Methods

Seven mongrel dogs were used; one of them was a puppy. All were healthy and remained so with the exception of dog No. 3, which developed a malignancy of the vulva. They were first fed a commercial diet (Purina Dog Chow Checkers, "Diet A") for a period of 10 weeks; 5 weeks for dog No. 4. Body weight and serum cholesterol were determined weekly. Following the control period, the dogs were put on a cholesterol-free diet ("Diet B") for 128 to 135

Table 1

Diet*	Ingredients	Analysis† Fat percent	Protein percent	Carbohydrate percent
"A" (Purina Dog Chow Checkers)	Dried meat, dried skim milk, puratene, cod liver oil, wheat germ, yellow corn grits, corn cereals, wheat cereal, vegetable fibre, blackstrap molasses, organic minerals, iodized salt.	5	24	50
"B"	Corn flakes, 30 percent wheat flakes, 27 percent soy bean flour, 20 percent peanut flour, 20 percent brewer's yeast, 2 perecent iodized salt, 1 percent ‡	13	20	55

*Both diets were furnished by Ralston Purina Company, St. Louis, Mo.
†Crude content.
‡Percent by weight.

* From the John Sealy Memorial Laboratory for Clinical Research and the Department of Medicine, The University of Texas, Medical Branch, Galveston. Received for publication February 27, 1954.

weeks. Body weight and serum cholesterol levels were observed weekly during the first nine to seventeen weeks, and again during the last forty weeks, of the cholesterol-free diet period. The composition of the control diet (Diet A) and the cholesterol-free diet (Diet B) is listed in table 1. We were informed by the manufacturer of "Diet A" that the cholesterol content was quite low. No exact figure could be quoted because no satisfactory method had been found for accurate measurement. The cholesterol-free diet ("Diet B") was completely vegetarian. In preparation of "Diet B", corn flakes, wheat flakes, soy bean flour and peanut flour were toasted and well mixed with yeast, salt and water; and then fed in the form of balls. "Diets A and B" were unrestricted in quantity. Cholesterol determinations were made with the revised Schoenheimer-Sperry method (Schoenheimer and Sperry, 1934; Sperry, 1945).

Results

The results of the control period on "Diet A" and those of the last forty weeks of the cholesterol-free period on "Diet B" are in table 2. The results of the first 9 to 17 weeks of the cholesterol-free period constituted part of a previous report (Futch, *et al.*, 1949) and are not quoted in detail here. As will be noted from table 2, the serum cholesterol showed great variation from dog to dog in the 6 normal dogs (Nos. 1, 2, 4, 5, 6, and 7). The range of average serum cholesterol level in 6 dogs for the 10 weeks control period was 34 mgs. per cent to 57 mgs. per cent for free cholesterol; 81 mgs. per cent to 154 mgs. per cent for cholesterol ester; and 115 mgs. per cent to 211 mgs. per cent for total cholesterol. The average free to total cholesterol ratio varied from 0.27 to 0.30. The serum cholesterol level of each individual dog also fluctuated markedly from week to week.

After institution of the cholesterol-free diet, the serum cholesterol level fell in all dogs. A gradual and steady fall was already noticeable during the first 9 to 17 weeks (Futch, *et al.*, 1949). In the last forty weeks of the cholesterol-free diet period, the serum cholesterol level appeared stabilized and showed no further drop. The maximum fall in serum cholesterol apparently occurred before the 88th week of "Diet B". The reduction in serum cholesterol involved both free and ester fractions. During the entire period of 128 weeks or more, the reduction in the average free cholesterol level was 28 per cent to 51 per cent of the average control values in the seven dogs; the reduction of the average cholesterol ester, 29 per cent to 46 per cent; and that of total cholesterol, 29 per cent to 46 per cent. In four dogs (Nos. 2, 3, 4, and

TABLE 2

Dog No.	Sex	Diet*	Body Wt. (kg.) Av.	Free cholesterol mg. percent Range	Free cholesterol mg. percent Av.	Cholesterol ester mg. percent Range	Cholesterol ester mg. percent Av.	Total cholesterol mg. percent Range	Total cholesterol mg. percent Av.	Free cholesterol total cholesterol ratio av.
1	F	A(10)	7.2	51–67	57	118–182	154	173–242	211	0.27
		B(128)	7.4	28–54	41	83–134	108	112–180	149	0.28
					–28%		–30%		–29%	
2†	M	A(10)	9.5	32–48	39	62–124	93	101–163	132	0.30
		B(135)	12.3	13–31	19	36–79	52	53–93	71	0.27
					–51%		–44%		–46%	
3‡	F	A(10)	13.9	32–43	37	77–103	91	105–146	128	0.29
		B(128)	10.4	21–42	27	56–96	69	79–125	96	0.28
					–27%		–24%		–25%	
4	M	A(10)	13.7	34–50	40	83–104	95	118–154	135	0.30
		B(128)	14.9	11–38	21	45–77	61	61–101	82	0.26
					–48%		–36%		–39%	
5	F	A(10)	13.2	32–59	46	85–129	111	121–183	157	0.29
		B(128)	14.2	12–36	25	40–99	60	55–134	85	0.29
					–46%		–46%		–46%	
6	F	A(10)	10.2	22–46	36	63–118	96	85–160	132	0.27
		B(132)	10.7	17–41	26	48–109	68	64–150	94	0.28
					–28%		–29%		–29%	
7	F	A(10)	10.4	32–36	34	74–87	81	110–121	115	0.30
		B(128)	10.5	8–32	21	41–80	56	60–109	77	0.27
					–38%		–31%		–33%	

*"Diet A"—Purina Dog Chow Checkers.
"Diet B"—Cholesterol free diet.
( )—Number of weeks of feeding.

†Growing dog.
‡Malignancy of vulva.
–%Percent decrease.

7) the fall in the free cholesterol was more marked than that of the cholesterol ester, thereby reducing the free and total cholesterol ratio. In the other three dogs (Nos. 1, 5, and 6) the fall in the free cholesterol was only slightly lower than, or equal to, that of the cholesterol ester. This tendency for the decrease in the free cholesterol to exceed that of the cholesterol ester seemed more apparent during the first nine to seventeen weeks of the cholesterol-free period. During that period the reduction in the average serum cholesterol level varied from 13 per cent to 23 per cent of the control values for free cholesterol and 11 per cent to 16 per cent for cholesterol ester.

Discussion. The wide variation of serum cholesterol in normal dogs on control "Diet A" observed here is comparable to that reported for the human subjects. The great fluctuation in serum cholesterol from week to week in each dog may indicate that cholesterol metabolism does not proceed at a constant rate. As mentioned, all the dogs except the one with malignancy (No. 3) remained in good health on the cholesterol-free diet. The only physical change noted during the cholesterol-free period was increased dental tartar. This can be easily explained by the soft character of "Diet B." Since all normal dogs in this series gained or maintained weight, the fall in serum cholesterol during the cholesterol-free period of feeding cannot be attributed to undernutrition. The fall in serum cholesterol in dogs after complete elimination of dietary cholesterol is similar to Key's observation in humans (Keys, 1950). It has often been assumed that the amount of cholesterol consumed is reflected in the serum cholesterol level and that reduction of the serum cholesterol follows diminution of cholesterol intake (Dock, 1950). Careful studies, however, indicate that this relationship is not so simple. Keys (1950) has demonstrated that serum cholesterol level in the human subject is independent of the cholesterol intake over a wide range and that the serum cholesterol falls only when the cholesterol intake approaches zero. As the cholesterol content in the control diet ("Diet A") is low, the significant fall in serum cholesterol after the cholesterol-free diet ("Diet B") supports Key's observation. Although the serum cholesterol falls with the cholesterol-free diet, doubt has been raised as to whether the elimination of exogenous cholesterol is the only factor which causes the reduction (Keys, 1950; Kinsell, *et al.*, 1953; Coch-

rane, *et al.*, 1953). The studies by Sperry and Bergman (1937), Peterson (1950), Kinsell (1953) and Cochrane (1953) have indicated that plant sterols (phytosterols) or some unidentified constituents in vegetable fat play an active role in lowering the serum cholesterol level. The plant sterols, even though not absorbed by the digestive tract (Sperry and Bergman, 1937), may not be inert in the control of cholesterol absorption. The mechanism of this action has, however, not been clarified. In this experiment, "Diet B" contains more than two and a half times as much fat as "Diet A" and the fat in "Diet B" is entirely of vegetable origin. The relationship of plant sterols in "Diet B" to the reduction of serum cholesterol in these dogs was not investigated. The determination of fecal cholesterol during the control period and the cholesterol-free period would have thrown some light on the postulate that plant sterols lowers the serum cholesterol by interfering with the absorption of exogenous and endogenous cholesterol from the intestinal tract (Sperry and Bergman, 1937; Peterson, 1950). We are not able to explain why the growing dog (No. 2) showed the most marked fall in serum cholesterol or why the fall was the least in the dog with malignancy (No. 3) following the cholesterol-free diet. The significance of the trend for the free cholesterol to decrease more than the cholesterol ester is likewise not clear.

Conclusion. It was demonstrated that dogs could be maintained in apparently good condition on a cholesterol-free diet for long periods. The dogs on the cholesterol-free diet showed a significant fall in both free and ester fractions of serum cholesterol.

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STUDIES ON THE ALKALINE AND ACID PHOSPHATASE CONTENT OF LEUKOCYTES IN VARIOUS HEMATOLOGICAL DISORDERS*

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The biochemical constituents and the enzymatic activities of leukocytes have been studied by a number of workers using either histochemical techniques or quantitative estimations (Valentine, 1951). It has been determined that glycogen, histamine and sulfhydryl-containing amino acids enter into the composition of leukocytes. A number of enzymes have been detected but of these only the phosphatases have been extensively studied. Haight and Rossiter (1950) found that alkaline phosphatase activity resides largely in the granulocytic leukocytes and acid phosphatase in the lymphocytes and to a small extent in the granulocytes. Wachstein (1946) has shown that the blast forms

TABLE 1

Alteration of Leukocyte Phosphatase Activity in Various Hematological Disorders According to Valentine and Beck

Hematological disorder	Leukocyte phosphatase activity	
	Alkaline	Acid
Acute leukemia	low	low
Chronic lymphatic leukemia	low	low
Chronic myeloid leukemia	low	normal to slightly elevated
Polycythemia vera with leukocytosis	markedly elevated	normal
Leukocytosis with infection	moderately elevated	normal
Leukocytosis without infection	slightly elevated	normal

are poor in phosphatases. Valentine and his group (Beck and Valentine, 1951; Valentine, *et al.*, 1951, 1952) have recently carried out a quantitative analysis of the acid and alkaline phos-

* From the Laboratory Service, Veterans Administration Center, Temple, Texas. Presented before the Section on Clinical Pathology at the Annual Session, 1953, of the Texas Medical Association.

phatase content of the leukocytes in a number of hematological disorders and in leukocytoses of infectious and noninfectious origin. These workers found that in a number of conditions, there is a characteristic disturbance in the leukocytic phosphatase content. Their results are summarized in table 1.

Valentine and his associates have also studied the glycogen and the histamine content of the leukocytes in these diseases and have found that these constituents also show characteristic and consistent aberrations. This group feels that all three biochemical parameters, phosphatases, glycogen and histamine, should be used in studying and evaluating bizarre hematological disorders.

As this work has great interest from both the theoretical and practical standpoint, confirmation of these findings by others seems desirable. Therefore we decided to study the phosphatase content of the leukocytes in several hematological disorders using the method described by Valentine and his group. For technical reasons, we have as yet carried out no studies on the other constituents.

Method and Materials

The method used is essentially that of Valentine and Beck (1951). Leukocytes are separated from fresh venous blood after the erythrocytes have been sedimented by the addition of bovine fibrinogen (Amour, Fraction I). The leukocytes are freed from plasma and platelets by differential centrifugation and washing and are then resuspended in saline. The final cell concentration of the suspension is determined by leukocyte counts done in quadruplicate. The phosphatase activity is determined by measuring the amount of phosphorus released [method of Fiske and SubbaRow (1925)] when the leukocytes are incubated with sodium β-glycerophosphate buffered at pH 9.9 for alkaline activity and at pH 5.0 for acid activity. Phosphatase activity is expressed as the milligrams of phosphorus liberated by 10^{10} leukocytes per hour. Valentine and his co-workers suggest a normal alkaline range of from 13.4 to 58.0 and a normal acid range of 13.7 to 36.8.

Experimental Results: Our series includes: three cases of acute myeloblastic leukemia, one of chronic myelogenous leukemia, one of chronic lymphatic leukemia, two of leukocytosis with infection, one of polycythemia vera with a marked leukocytosis, one of myelofibrosis with either supervening or concurrent myeloid hyperplasia of the bone marrow, one of refractory anemia terminating with myelogenous leukemia and thirteen controls. The results of our studies are shown in table 2 and table 3. The three cases of acute myeloblastic leukemia showed low alkaline and acid phosphatase, and the case of chronic myeloid leukemia showed low alkaline and normal acid

phosphatase activity. Those with leukocytosis due to infection had elevated alkaline phosphatase and normal acid phosphatase activities. Both types of enzyme activity were depressed in the case of chronic lymphatic leukemia.

TABLE 2

Leukocyte Phosphatase Activity in Several Hematological Disorders

Patient		Diagnosis	Phosphatase activity (mg. P/10^{10} leukocytes/hr.) Alkaline	Acid
FJ		Acute leukemia	1.4	4.2
RO		Acute leukemia	6.7	2.6
CS		Acute leukemia	7.3	8.6
LW		Chronic myelogenous leukemia	7.1	14.0
SS		Chronic lymphatic leukemia	5.5	5.7
HH		Leukocytosis with infection	155.0	33.6
TG		Leukocytosis with infection	96.4	32.1
BO	1	Polycythemia Vera	78.1	36.8
	2	(after 2 months)	102.4	19.4
	3	(after 4 months)	89.0	18.9
	4	(after 6 months)	53.9	13.9
	5	(after 7 months)	39.4	14.9
AF		Myelofibrosis	3.4	16.4
SA	1	Refractory anemia terminating with myelogenous leukemia	4.4	31.0
	2		6.4	34.4
Normal Range according to Valentine and Beck			13.4–58.0	13.7–36.8

In the case of polycythemia vera there is a persistent marked leukocytosis with considerable immaturity of the cells. The white counts range between 70,000 and 158,000 per cu. mm. with from 2 to 40 per cent blast forms. This patient has been followed for seven months and during this time five phosphatase estimations have been performed. The first three estimations show marked elevation of the alkaline phosphatase activity with a normal acid activity. The alkaline phosphatase has now fallen to within the normal range and concurrently the leukocytosis and the myeloid immaturity in the peripheral blood have become more pronounced.

Our case of myelofibrosis was admitted with splenomegaly, normocytic anemia and leukocytosis (21,000 cells per cu. mm.) and hypertensive cardiovascular disease. A biopsy of the sternal marrow showed extensive fibrosis. Subsequently both the leukocytosis and the anemia became more marked and

a few myelocytes appeared in the peripheral blood. Six months later the sternal marrow was again examined and myeloid hyperplasia was found. Shortly thereafter this patient came to autopsy and widespread extramedullary hematopoiesis was demonstrated but no leukemic infiltrations of the organs could be detected. Unfortunately the marrow was not examined at the autopsy. The only phosphatase estimation performed was carried out after the hyperplasia of the marrow had been detected. It was found that the alkaline phosphatase activity was low and the acid activity normal, the pattern found in chronic myeloid leukemia.

TABLE 3

Leukocyte Phosphatase Activity in Control Subjects

Subject		Phosphatase Activity (mg.P/10^{10} leukocytes/hr.) Alkaline	Acid
RR		33.1	27.2
JM		20.5	20.0
MC		17.6	20.1
DG		35.3	14.1
HH		18.1	24.9
JC		18.7	20.8
BY		28.1	23.2
MA		25.1	18.2
CO		39.0	33.6
GN		17.9	15.5
HL		13.8	27.5
ES		16.1	14.3
MV	1	2.8	25.8
	2	2.7	36.2
Normal Range according to Valentine and Beck		13.4–58.0	13.7–36.8

The last case in our series is hard to evaluate. This patient suffered with a refractory anemia, leukopenia and thrombocytopenia for 8 years except for a 12-month period of complete remission which occurred about one and a half years after the onset of the disease. During the initial period, the marrow showed panhypoplasia. After the relapse there was at first a similar hypoplasia but later erythroid hyperplasia ensued and, finally, myeloid hyperplasia with considerable immaturity. Two phosphatase estimations were performed, both during the final phase. The phosphatase activities were compatible with chronic myelogenous leukemia, while morphological studies of the peripheral blood and of aspirated marrow suggested acute myelogenous

leukemia. At autopsy widespread extramedullary hematopoiesis was found, and leukemic infiltrations were present in both the liver and the spleen. In addition, hemachromatosis, which was considered to be exogenous since this patient had received 150 transfusions, was present.

In the study of our thirteen controls, one disconcerting individual has been encountered. Despite both a normal differential and total leukocyte count and a completely asymptomatic clinical picture, his phosphatase studies present the pattern associated with chronic myelogenous leukemia. The leukocytic alkaline and acid phosphatase activities of the other twelve individuals fall in the normal range.

Comment. Our results confirm the work of Valentine and his group that characteristic aberrations of the acid and alkaline leukocytic phosphatase content occur in certain hematological disorders such as acute leukemia, chronic myelogenous leukemia, chronic lymphatic leukemia and leukocytosis with infection. These results suggest that the leukocytes in these diseases vary biochemically from their normal counterparts. For a more thorough evaluation, the histamine and the glycogen content of the leukocytes should also have been studied, but at the present time we have not made these estimations. It must be emphasized that all these values are expressed in terms of 10^{10} leukocytes and thus the leukocyte count per cu. mm. has no bearing upon the results. As blast cells are deficient in both alkaline and acid phosphatase (Wachstein, 1946), these activities are low when the blast forms predominate among the leukocytes in the peripheral blood. Similarly as the granulocytic series is the repository of alkaline phosphatase, the level of this enzyme is depressed when lymphocytes predominate.

Our cases of myelofibrosis and polycythemia vera are of particular interest and are worthy of more detailed consideration. There has been much debate as to the frequency with which leukemia supervenes in polycythemia vera (Merskey, 1949). Dameshek is a proponent of the theory that marked leukocytosis with immature cells is usually a benign phenomenon and a manifestation of splenic myeloid metaplasia in nonirradiated cases (Dameshek, 1950). Valentine and his associates (1952) have studied a series of 21 cases of polycythemia vera. Four of these had leukocytoses ranging from 40,000 to 80,000 per cu. mm. with cellular immaturity and marked splenomegaly and carried tentative diagnoses of polycythemia vera with chronic myelogenous leukemia. In all 21 cases, the alkaline phosphatase

and the glycogen content of the leukocytes were considerably increased. However, in the 4 cases with the marked leukocytosis, the histamine content was also abnormal and approached that found in chronic myelogenous leukemia suggesting some kinship with the leukocytes in that condition.

In our case, the leukocytic alkaline phosphatase activity remained consistently elevated over a period of four months. The last two estimations performed during the 6 and 7 months have revealed a progressive depression of this activity into the normal range, while the leukocytosis and the cellular immaturity have become more pronounced (see Table 3). This change in phosphatase activity may indicate incipient transition to leukemia and the alkaline phosphatase content may eventually fall in the range compatible with that disease. The patient is still being followed and it is hoped that further studies will determine whether a leukemoid reaction has been succeeded by leukemia with concomitant changes in the leukocytic phosphatases.

It must be borne in mind, when evaluating these studies, that the blast forms are poor in phosphatases and as the percentage of these cells increase the alkaline phosphatase per 10^{10} leukocytes will decrease. This patient has received no treatment apart from phlebotomies and a low iron diet and has at no time been irradiated.

Valentine and his group (1952) studied 7 cases of idiopathic myelofibrosis associated with myeloid metaplasia of the spleen. These were not considered to be examples of "spent" cases of polycythemia vera. Three of these 7 cases had leukocytic phosphatase activities compatible with chronic myelogenous leukemia, but only 1 of these had similarly deranged histamine and glycogen levels. Studies on the other 4 cases in the group revealed leukocytic biochemical abnormalities somewhat suggestive but not completely typical of polycythemia vera. In our case of idiopathic myelofibrosis the single estimation of the leukocytic phosphatases showed them to be compatible with chronic myelogenous leukemia. As already mentioned, this determination was performed late in the course of the disease and after the marrow had been found to show marked myeloid hyperplasia rather than fibrosis. Thus it is not known whether the leukocytes also had the biological characteristics of this type of leukemia at the time that the marrow was found to be fibrotic.

It is suspected however that the marrow contained both fibrotic and hyperplastic areas throughout the period of observation and that, at the two examinations, different areas were encountered. Unfortunately the marrow was not examined at autopsy and there is no confirmation for this supposition. It must be recalled that, apart from a few myelocytes, immature leukocytes were not present in the peripheral blood and at no time were blast forms encountered.

Custer (1949) maintains that idiopathic myelofibrosis is but a stage in the evolution of chronic myelogenous leukemia. Others, however, believe that leukemia is usually only simulated in this condition (Carpenter and Flory, 1941; Jackson, *et al.*, 1940; Vaughn and Harrison, 1939; Wyatt and Sommers, 1950). Biochemical studies on the leukocytes suggest that this condition is not a single entity but a heterogeneous group, with some cases having a closer relationship to chronic myelogenous leukemia than others.

Dameshek (1951) has classified together under the title of myelo-proliferative disorders such maladies as chronic myeloid leukemia, polycythemia vera, idiopathic myeloid metaplasia of the spleen, megakaryocytic leukemia and erythroleukemia, and has suggested that they may represent variable reactions to a single myelo-stimulatory factor. Valentine and his associates (1952) have suggested that in this group of disorders various distinct metabolic defects may produce morphologically similar but metabolically different entities. The data, which these workers have presented and which we have in part confirmed, corroborate this thesis.

The finding of leukocytic phosphatase activity compatible with chronic myeloid leukemia in one of our "controls" cannot be explained. It is interesting to speculate whether or not this change represents a preleukemic phase of leukemia. It has not been possible to examine the marrow of this individual, but it is hoped that we will be able to keep him under observation and to study further the biochemistry of his leukocytes.

We have presented the results of our study of the leukocytic phosphatases in several hematological disorders in the hope that they will stimulate others to carry out similar investigations. Only by the accumulation of data from a number of laboratories can the significance of these biochemical variations of

leukocytes in certain disorders be evaluated. If further confirmation of the work of Valentine and his group becomes available, it is possible that studies along these lines may eventually have considerable value in the delineation of the myeloproliferative group of diseases.

Summary. The biochemical studies on leukocytes by Valentine and his group have been briefly reviewed. Using the method devised by these workers, we have studied the leukocytic phosphatase activity in 3 cases of acute leukemia, 1 case of chronic myeloid leukemia, 1 case of chronic lymphatic leukemia, 2 cases of infectious leukocytosis, 1 case of polycythemia vera with marked leukocytosis, 1 case of idiopathic myelofibrosis, 1 case of atypical refractory anemia terminating with myelogenous leukemia, and 13 so-called normal individuals. The results obtained from our investigation corroborate those presented by Valentine and his group. The possible significance of these results is briefly discussed and it is suggested that further study along these lines may do much to elucidate the interrelationships of myelo-proliferative diseases.

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VARIATIONS WITH AGE OF CULTURE IN THE ABILITY OF *TETRAHYMENA GELEII (Y)* TO OXIDIZE AND TO "INCORPORATE" METHIONINE

W. J. Wingo and L. E. Cameron*

Many studies have been made of the response of the ciliated protozoan, *Tetrahymena geleii*, to variations in composition of its nutrient medium, with the result that several strains of this organism can now be grown in completely or nearly completely synthetic media. Valuable as these studies are, however, in most of them the cell itself has been used as a test object, and its response has been determined primarily by measurements of the reproduction of the cells. On the other hand, it has been established by Seaman (1951) that many of the enzymes found in higher organisms also occur in this ciliate, and that its overall metabolic pattern is much the same as that of the usual laboratory mammals.

In common with other microorganisms, *T. geleii* exhibits growth rates which vary widely—generation times on the order of three hours are not uncommon during the early phases of the growth of a culture, and much slower rates of reproduction occur later, when exhaustion of the limiting nutrient is incipient (Browning, Lockingen, and Wingo, 1952).

The analogies of the rapidly growing, young cultures to malignant or embryonic cells and of the slowly growing, older cultures to "normal" cells are most attractive. It is of interest to compare in various ways the metabolic potentialities of cells taken from these two phases of a culture, but studies of this sort are few. Studies on the oxygen uptake of *T. geleii* have indicated that young cultures respire more rapidly than older ones (Pace and Lyman, 1947; Baker and Baumberger, 1941; Wingo and Cameron, 1952), and that the stimulation of respiration by thyroxin present in the medium decreases both absolutely and relatively with age of culture (Wingo and Cameron).

The present investigation represents an attempt to extend work of this sort.

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Experimental. Bacteria-free cultures of *T. geleii (Y)* were grown in the culture medium of Wingo and Cameron. The medium was dispensed, in two-liter lots, in three-liter all-glass flasks equipped with sampling spouts, inoculation ports, and aeration tubes with fritted-glass air dispersers. After inoculation the cultures were incubated in a water bath at 37° C.

The growth of the cultures was followed by removing samples, fixing the cells with formaldehyde, and making direct counts using a Segewick-Rafter cell and a microscope fitted with a Whipple eyepiece micrometer.

When the culture density had reached the desired value (10^4 cells or more/ml.) a sample of the culture sufficient to contain 5×10^6 or more cells was removed and chilled to 3°–4° C. to stop ciliary action. The cells were harvested by centrifuging gently (under conditions which sedimented the cells without causing noticeable displacement of their nuclei) and were resuspended in a total volume of 55 ml. Samples were removed for counting (the average length and breadth of the cells in the sample were determined by measuring 12 or more cells selected at random, and the volume of protoplasm per ml. calculated on the assumption that the cells were prolate ellipsoids of revolution).

Fifty ml. of the concentrated culture was placed in a flask of the type described by Browning, Brittain, and Bergendahl (1952) which contained 1 mg. of DL-methionine labeled with S^{35}.[1]

The culture-methionine mixture was incubated, under aeration, for two hours at 37° C.; then the cell suspension was again chilled, and the cells were harvested by vigorous centrifuging.

An aliquot of the supernatant was heated with HCl under the general conditions of Folin (1905) with the addition of a small quantity of Darco decolorizing carbon, and the carbon was filtered off and washed. A suitable amount of carrier was added to the pooled filtrate and washings and the total sulfate was precipitated, filtered, mounted, and counted for radioactivity by the methods previously used in this laboratory (Wingo, Smith, and Wood, 1954).

The cells were homogenized with trichloroacetic acid, and the T.C.A. precipitate was washed with trichloroacetic acid and with acetone (Winnick, Friedberg and Greenberg, 1947). The precipitate was washed once with ether, dried, and dissolved in 0.5M sodium hydroxide solution. The organic matter in aliquots of this solution was oxidized by fusion with a mixture of sodium carbonate and sodium peroxide (Bailey, 1937). The fusion mixtures were leached with water and the solution prepared for counting in the usual manner. Standards for counting were prepared by Bailey fusion of aliquots of the stock labeled methionine solution, addition of carrier, and subsequent treatment as in the case of the samples.

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[1] The labeled methionine was obtained under A.E.C. allocation from Abbott Laboratories, Inc., North Chicago, Illinois. The desired quantity was weighed out, and sufficient inactive methionine was added to make 10 mg. The total sample was dissolved in water and diluted to 10 ml., and 1 ml. samples were dispensed into the flasks. The flasks were placed in a desiccator over silica gel, and the desiccator was evacuated to evaporate the water from the methionine.

The radioactivities of standards and samples were determined by counting, using an end-window G. M. counter (window 2 mg./cm.²) to a statistical accuracy of better than 2 per cent. Appropriate corrections for self-absorption were made, and from these and the cell count and size data the per cent of the sulfur of the labeled methionine (a) oxidized to sulfate and (b) incorporated into the T.C.A. precipitable protein were computed.

The entire procedure was repeated later when the growth rate of the culture had appreciably declined; in some cases a third sample was taken during the "stationary" period.

Results

The results obtained in two typical experiments are presented in figure I and figure II.

It is evident that under the conditions employed the extent of oxidation of methionine sulfur to sulfate is less in the cells of older, slowly-growing cultures than in the cells of younger, rapidly-growing cultures. Similarly, the ability of the cells to incorporate this amino acid into protein decreases as the age of the culture increases (and its growth rate declines).

Discussion. Friedberg, Schulman, and Greenberg (1948) reported that the decrease in rate of growth of rat liver tissue which occurs with increasing age is accompanied by a marked decrease in the ability of liver homogenates to incorporate labeled glycine into their proteins. Zamecnik, Frantz, Loftfield and Stephenson (1948) found that rat hepatoma slices incorporated labeled alanine and glycine more rapidly than slices of normal liver or of the apparently normal portions of hepatomatous liver. The results of the present study (as regards incorporation of methionine into the protein of *T. geleii*) thus show a similarity of behavior of cells from young cultures to embryonic and to at least some types of tumor cells in that such cells incorporate methionine more rapidly than do cells in a later stage of the culture when the rate of growth has declined. This finding is, of course, in accord

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FIGURES I AND II

Effect of age of culture (growth rate) on incorporation of methionine into T.C.A.-precipitable proteins of *T. geleii (Y)* and on oxidation of methionine sulfur to sulfate by *T. geleii.*

(The points represent the populations found in the cultures at the times given; the curves are smooth fits (by eye) of these points. The bars represent the logarithms of the per cent of sulfur oxidized to sulfate (striped) and incorporated into the T.C.A. precipitable proteins (stippled) at the time shown by the line between each of each pair of bars.)

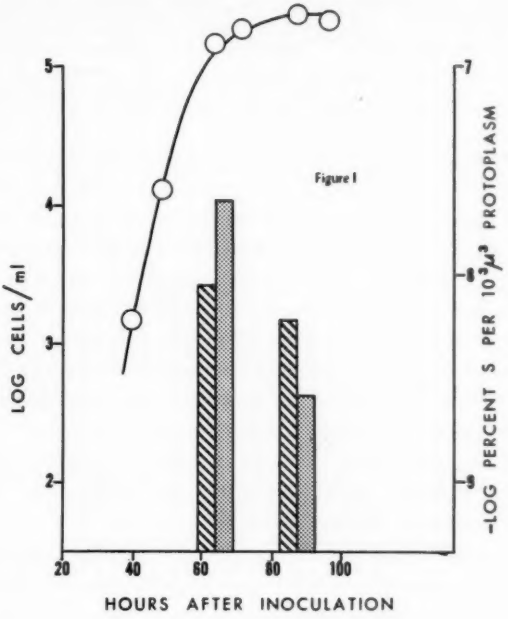
Figure I
LOG CELLS/ml
-LOG PERCENT S PER $10^3\mu^3$ PROTOPLASM
HOURS AFTER INOCULATION

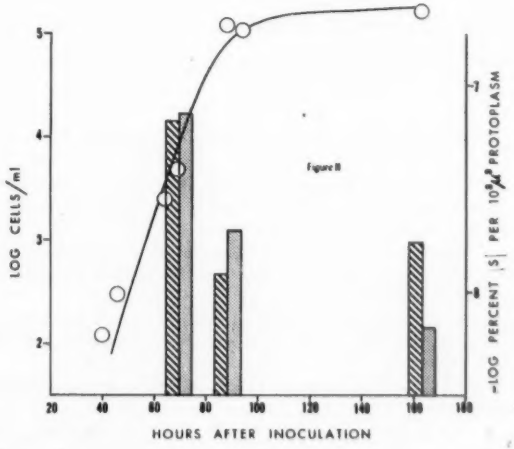
Figure II
LOG CELLS/ml
-LOG PERCENT S PER $10^3\mu^3$ PROTOPLASM
HOURS AFTER INOCULATION

with expectations, since active division of cells must be accompanied by formation of new protein.

The incorporation of amino acids into protein of fetal homogenate (Winnick, 1950) or of lymphosarcoma cells (Kit and Greenberg, 1951) has been shown to be a process requiring the expenditure of energy. It has been shown previously that the respiratory rate of *T. geleii* decreases with increasing age of culture; the decrease in energy available to the cell incident to the decline in rate of respiration may influence the rate of amino acid incorporation by the cell; certainly the changes in this function are in the right sense for this to be true. The rate of oxidation of methionine sulfur to sulfate declines in the same sense; the secondary increase shown in figure II may represent increased utilization of the added methionine by organisms surviving in a medium considerably depleted in nutrients.

Summary. Cells from young cultures of *T. geleii (Y)* incorporate more methionine into protein than do cells from the same cultures after growth has slowed.

Cells from young cultures oxidize the sulfur of methionine to sulfate more rapidly than do cells from the same cultures after growth has slowed. (A partial reversal of this trend may occur later).

The significance of these findings is discussed in relation to normal and malignant growth.

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CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

February, 1954

Clinical: *HB Atlee discusses Chronic Iliac Pain in Women* (CC Thomas, Springfield, Ill, 54, 65pp, $2.5) . . .AL Barach & GJ Beck recommend head down position for relief in pulmonary empysema (*Am J Med* 16/55/54) . . . FC Combs describes *Coal Tar & Cutaneous Carcinogenesis in Industry* (CC Thomas, Springfield, Ill, 54, 75pp, $2.5). ME DeBakey analyses thromboembolism (*SGO Internat Abs Surg* 98/1/27/54 . . . DD DeWeese offers *Dizziness: An Evaluation & Classification* (CC Thomas, Springfield, Ill, 54, 80pp, $2.5) . . . DL Fry & Co. review machanisms of pulmonary ventilation in health & in emphysema (*Am J Med* 16/80/54) . . . MR Harrower & R Herrmann suggest *Psychological Factors in the Care of Patients with Multiple Sclerosis: For Use of Physicians* (National Multiple Sclerosis Soc, NY, 53, 32pp) . . . CP Rhoads discusses rational cancer chemotherapy (*Science* 119/77/Jan 15/54) . . . FE Russell reviews treatment of sting-ray injuries (*AM J Med Sci* 226/611/63) . . . WC Stadie surveys insulin action (*Physiol Rev* 34/53/54) . . . JC White discusses conduction by afferent paths in cord & visceral nerves (*Arch Neurol Psychi* 71/1/54) . . . LL Wiesel & AS Barritt show p-amino benzoic acid aids cortisone therapy by delaying cortisone destruction (*Am J Med Sci* 227/74, 80/54) . . . F Wyss & J Regli analyse problem of cardiac dyspnoea (*Schw Med Wchnschr* 83/1175/Dec 5/53).

Preclinical: HB Chase reviews hair growth (*Physiol Rev* 34/113/54) . . . RJ Dubos & JG Hirsch note thymus-peptide inhibition of mycobacteria (*J Exp Med* 99/55,65,79/54) JM Garfunkel & Co show direct relation between oxygen consumption & cerebral function (*J Pediat* 44/64/54) . . . B Grad & Co find eosinopenic test satisfactory for assay of adrenal cortical steroid activity (*Proc Soc Exp Biol Med* 84/1/53) . . . D Granaat explores spleen in regulation of arterial blood pressure (*J Physiol* 122/209/53) . . . AG Hills & Co discuss adrenal cortical regulation of water & electrolyte distribution in humans (*J Clin Invest* 32/1236/53). . . . J Kieler discusses influence of amino acids on mitotic activity in fibroblast culture (*Acta Path Microbiol Scand* 33/337/350, 361/53) . . . E Kovacs describes nucleases in cerebrospinal fluid (*Canad J Med Sci* 31/437/53) . . . EK Marshall & WF Fritz find alcohol in low conc in blood disappears at rate proportional to amount present, but at high conc at rate independent of amount (*J Pharmacol* 109/431/53) . . . E Mohme-Lundholm shows relaxing effect of adrenaline on smooth muscle is due to lactic acid formation (*Acta Physiol Scand* 29, Suppl 108, 53, 61pp) . . . K Neumann & G Koch demonstrate localization of succinic dehydrogenase by tetrazol purple (*Hoppe-Syeler's Ztschr Physiol Chem*, K Thomas Festschrift, 295/35/53) . . . MK Paasonen & TE Peltmen note increased tolerance to epinephrin in pregnancy (*Ann Med Exp Biol Fenn* 31/275/53) . . . NT Spratt Jr notes physiological mechanisms in development (*Physiol Rev* 34/1/54) . . . R Stampfli describes saltatory conduction

in nerve (*ibid* p 101) . . . JH Stover Jr. & Co indicate botulinum toxin inhibits acetylcholine release at motor end plates (*Proc Soc Exp Biol Med* 84/146/53) . . . MK Streifeld & MS Saslaw describe skin test for Rheumatic activity in children (*ibid* p 628) . . . M Taubenhaus & Co note inhibiting effect of dibenamine & banthine on granulation, like cortisone (*ibid* p 646).

GENERAL: Sir Edward Appleton appeals for science for its own sake (*Science* 119/103/Jan 22/54) . . . DW Atchley brings healer & scientist together in ideal full-time clinical teacher (*Sat Rev* Jan 9/54) . . . Lee T'ao describes achievements of Chinese medicine in Ch'in & Han periods (221–119BC) (*Chinese Med J* 71/380/53) . . . VF Lenzen discusses *Causalty in Natural Science* (CC Thomas, Springfield, Ill, 54, 121pp, $3). RR Loehrich offers remarkable philosophical analysis of semantics, values, dreams, psychosomatic ills, & interpersonal relations (*Oneirics & Psychomatics: An Introductory Treatise Concerning a New Theory of Psychoanalysis, Its Logic & Methodology*, Compass Press, McHenry, Ill, 171pp) . . . EO Lippmann offers *Beitrage zur Geschichte der Naturwissenschaften und der Technik* (Verlag Chemie, Weinheim, 53, 2 vols, 380, 369pp, DM 45) . . . JC Moloney notes paradox of Japanese psychoanalysis where all individuals are merely part of the State (*Internat J Psychoanal* 34/291/53) . . . GK Plackmann edits *The Resources of Leonardo da Vinci* (Southern Illinois Univ, Carbondale, 53, 39pp) . . . T Reik offers *The Haunting Melody: Psychoanalytic Experiences in Life & Music* (Farrar Straus, NY, 53, 376pp, $4) . . . EA Underwood edits *Science Medicine & History: Essays on the Evolution of Scientific Thought & Medical Practice Written in Honour of Charles Singer* (Oxford Univ Press, London, 54, 2 vols, 564 & 646pp, $45).

MARCH 1954

GENERAL: DW Bronk evokes adventuring spirit in furthering science (Science 119/223/Feb 19/54), and EV Condon emphasizes the duty of dissent (*ibid.* p 227) . . . WR Catton Jr. finds human values are measurable relative to each other in accordance with Thurstone's principle of comparative judgment (*Am Soc Rev* 19/49/54) . . . BC Corner tells about *William Shippen Jr Pioneer in American Medical Education* (American Philosophical Soc, 104 S 5th, Phila, 54, 161pp, $2.75) . . . F Daniels edits *The Challenge of Our Times* (Burgess Publ Co, Minneapolis 15, 54, 364pp, $3.50) . . . AE Emerson describes dynamic homeostasis as a unifying principle in social and ethical evolution (Sci. Month., 78/67/54) . . . A Huxley suggests mescalin as a socially acceptable (?) escape agent, but too little known of action or long range effects (*The Doors of Perception*, Harper's, NY, 54, 79pp, $1.5) . . . OE Klapp considers significance of group categorizing of heroes, villains & fools to make them agents of social control (*Am Soc Rev* 19/56/54) . . . HP Laughlin suggests psychological approach to executive development (*Dis Nerv Sys* 15/12/54 . . . L Linskey edits *Semantics and the Philosophy of Language* (Univ of Illinois Press, Urbana, 54, 218pp, $3.5) . . . G Legman gives *Rationale of the Dirty Joke* (Schuman, NY, 54, 450pp, $6) . . . CD O'Malley translates & annotates the writings of *Michael Servetus* (American

Philosophical Soc, Phila, 54, 208pp, $3) . . . FC Neff deplores the retreat from heresy (*Sci Month.*, 78/19/54) . . . H&B Overstreet enjoy *The Mind Alive* (WW Norton, NY, 54, 268pp, $3.75) . . . W Penfield novelizes about Abraham & Sarah in *No Other Gods* (Little Brown, Boston, 54, 351pp, $3.5). . . . HW Smith goes renally *From Fish to Philosopher* (Little Brown, Boston, 54, 264pp, $4). GS Stevenson edits *Conferences on Administrative Medicine* (Josiah Macy Jr Foundation, NY, 54, 180pp, $3) . . . LG Stevenson describes *Nobel Prize Winners in Medicine & Physiology* (Schuman, NY, 54, 480pp, $6.5) . . . H Taylor essays *On Education & Freedom* (Schuman, NY, 54, 250pp, $3.5) . . . M Thompson shows he comes to medicine *Not As a Stranger* (Scribner's, NY, 54, 948pp, $4.75) . . .

PRECLINICAL: LG Aboord shows electrical impulses inhibit phosphorylation in brain mitochondria (*Am J Physiol* 176/247/54) . . . JC Eccles discusses *The Neurophysiology of Mind* (Oxford Univ Press, NY, 54, 314pp, $6.5) . . . E Eichenberger & Co. note fibrinolytic action of thrombin (*Schw Med Wchnschr* 84 65/Jan 10/54, Festschr to W Fry . . . GJ Fruhman and Co find somatotrophin increases red cell formation (*Proc Soc Exp Biol Med* 85/93/54) . . . E Gellhorn & Co assume interaction between hypothalamic cortical system & a specific cortical projection area is physiologic basis of sensation & perception (*J Neurophysiol* 17/14/54) . . . SE Luria covers *General Virology* (Wiley, NY, 54, 427pp, $8.5) . . . JP Marbarger edits Space Medicine: *The Human Factor in Flights Beyond the Earth* (Univ. of Illinois Press, Urbana, 54, 210pp, $3) . . . W. Menaker finds Mg requirements for protein metabolism increases with protein synthesis and intake (*Proc Soc Exp Biol Med* 85/149/54) . . . J Moshman & AC Upton propose depigmentation of hair as biological radiation dosimeter (*Science* 119/186/Feb 5/54). . . . H Quastler develops *Information Theory in Biology* (Univ Illinois Press, Urbana, 54, 256pp, $4) . . . G Scholes & J Weiss show chemical action of x-rays in splitting nucleic acids. (*Biochem J* 56/65/54) . . . WH Sebrell & RS Harris edit *The Vitamins: Chemistry, Physiology, Pathology* (Academic Press, NY, 54, 3 vols, 750pp each, $49) . . . CE Shannon & W Weaver offer *The Mathematical Theory of Communication* (Univ Illinois Press, Urbana, 54, 118pp, $2.5) . . . AD Wallis thinks streptolysin S is etiologic factor in rheumatic fever & can be neutralized by choline in eggs (*Am J Med Sci* 227/167, 171/54) . . .

CLINICAL: W Bartsche-Rochaix discusses physiopathology of migraine prodrome (*Schw Med Wchnschr*, W Fry Festchrift, 84/51/Jan 10/54) . . . MA Hayes discusses shock & the adrenocortex (*Surg* 35/174/54) . . . MR Hilleman and JH Werner isolate new virus from throats of patients with primary atypical pneumonia (*Proc Soc Exp Biol Med* 85/183/54) . . . IJ Hirsh offers *The Measurement of Hearing* (McGraw-Hill, NY, 54, 364pp, $6) . . . HV Ingham & LR Love describe *The Process of Psychotherapy* (McGraw-Hill, NY, 54, 270pp, $5) . . . H Racher discusses the problem of countertransference (*Internat J Psychoanal* 34/313/53) . . . A Sorsby considers *Clinical Genetics* (CV Mosby, St Louis, 54, 603pp, $17.5) . . .

APRIL, 1954

CLINICAL: EK Ahvenainen & A Ylinen suggest intrauterine trauma as factor in torticollis (*Ann Med Int Fenn* 43/6/54) . . . GR Ancona & IC Schumacher use frozen raw foods in skin testing for allergic disorders (*Calif Med* 80/181/54) . . . J Buchi reviews new developments in antibiotics as drugs (*Antibio Chemoth* 1/1/54) . . . JWL Doust proposes assessment of profile of emotional immaturity (*Am J Psychi* 110/651/54) . . . EL Dubois describes early cytologic diagnosis of systemic lupus erythematosus (*Calif Med* 80/154/54) . . . RR Grinker & FP Robbins offer *Psychosomatic Case Book* (Blakiston, NY, 54, 360pp, $6.5) . . . A Grollman discusses *Acute Renal Failure* (CC Thomas, Springfield, Ill, 54, 96pp, $2.5) . . . FW Hartman & Co edit *The Dynamics of Virus & Rickettsial Infections* (Blakiston, NY, 54, 461pp, $7.5). JS Hirschboeck & Co find alopecia, epistaxis, diarrhoea, & brittle nails may follow use of heparin or heparinoids (*Am J. Med Sci* 227/279/54) . . . HV Ingham & LR Love describe *The Process of Psychotherapy* (McGraw-Hill, NY, 54, 270pp, $5) . . . E. Kerpel-Fronius & Co show low kidney clearances in dehydration, but not in uncomplicated malnutrition (*Acta Med Acad Sci Hung* 5/27/54) . . . J Lequime & H Denlin discuss physiopathology of mitral stenosis (*France Med* 17/Feb 54/5) . . . ML Lowry finds insulin & jrotein-free pancreatic extract ("Depropanex") is beneficial in multiple sclerosis & suggests MS is due to carbohydrate metabolism disorder (*Am J Med Sci* 227/259/54) . . . JH Moyer & Co note chlorpromazine non-toxic antemetic & hypotensive agent (*ibid* p 283, confirmed by EM Boyd & Co (*Canad Med J* 70/276/54) . . . EJ Pulaski considers *Surgical Infections* (CC Thomas, Springfield, Ill, 54, 315pp, $6) . . . NB Strydom shows influenza, skin disorders, alcohol, & high work rate dispose toward heat stroke (*South Afr Med J* 28/112/Feb 6/54) . . . CA Whitaker & TP Malone dig *The Roots of Psychotherapy* (Blakiston, NY, 54, 236pp, $4.5).

GENERAL: I Berlin interprets history in *The Hedgehog & The Fox* (Simon &Schuster, NY, 54, 133pp, $2.5) . . . H Brown is Malthusianly provocative in *The Challenge of Man's Future* (Viking, NY, 54, 290pp, $3.75) . . . S Chase emphasizes *Power of Words* (Harcourt, Brace, NY, 54, 308pp, $3.95) . . . N Cousins well asks *Who Speaks for Man?* (Macmillan, NY, 54, 228pp, $3.5) . . . W Gibson spins fine psychological yarn in *The Cobweb* (AA Knopf, NY, 54, 369pp, $3.95) . . . RS Gill & Co eulogize Emil Novak at 70 (*Obs Gyn Surv* 9/1–155/54) . . . G Highet salutes *Man's Unconquerable Mind* (Columbia Univ Press, NY, 54, 138pp, $2.75) . . . G Piel opens debate on *The Scientist in American Society* (*Sci Month* 78/129–141/54) . . . H Schnitzler sternly debunks "gay" Vienna (*J Hist Ideas* 15/94/54) . . . WL Straus Jr describes the Piltdown hoax (*Science* 119/265/Feb 26/54) . . . H Taylor develops philosophy of intellectual freedom & moral values *On Education & Freedom* (Abelard-Schumann, NY, 54, 320pp, $4 . . . H Watkins relates how *Time Counts: The Story of the Calendar* (Philosophical Library, NY, 54, 226pp, $4.75).

PRECLINICAL: D Benitez & Co find fluoroacetate increases ammonium ion in brain with tendency to conculsions (*Am J Physiol* 176/488/54) . . . HD

Bouman edits symposium on ultrasonics in medicine (*Am J Phys Med* 33/1–91/54) . . . DM Donaldson & S Marcus show whole body x-irradiation depresses bactericidal action of blood (*J Immunol* 72/203/54) . . . RI Dorfman & F Unger discuss "*Metabolism of Steroid Hormones* (Burgess Publ, Minneapolis, 54, 238pp, $4) . . . H Eyer reviews chemical & physical aspects of viruses with 405 references (*Zntrl Bact Parasit Infekt Hyg* 160/98/54) . . . Federation Proceedings for Atlantic City meetings include 1721 abstracts (*Fed Proc* 13/1–525/54) . . . CA Handley & JH Moyer find dibenzazepine derivative, "Ilidar," hypotensive without blocking renal function (*J Pharmacol* 110/277/54) . . . DM Jackson & Me Nutt show swelling of red cells with increasing CO_2 tension (*J Physiol* 123/367/54) . . . K Krnjevic confirms opinion that peripheral nerve connective sheath is barrier to electrolute diffusion (*ibid* p 338) . . . A Loeser & Co show diethyleneglycol has toxicity like glycerine (*Arch Exp Path Pharmakol* 221/14/54) . . . SW Levy & RI Swank suggest heparin aids in fat transport & metabolism (*J Physiol* 123/301/54) . . . SM Moeschlin & Co show agranulocytosis of anaphylactic origin occurs by agglutination of circulating leucocytes (*Acta Haematol* 11/73/54) . . . KH Shull & Co find no atherosclerosis in dogs given high cholesterol diets (*Am J Physiol* 176/475/54) . . . J Whitney & Co note antidiuretic action of renin (*ibid* p 419).

MAY, 1954

ASSOCIATION PROCEEDINGS: 300 reports, 52 demonstrations, & 11 movies were presented at the meeting of the American Association of Anatomists at the Univ of Texas Medical Branch, Galveston, April 7, 8 & 9, 1954 (*Anat Rec* 118/275–443/54), & 23 reports were offered at the Tissue Culture Association session, April 6, with many informal demonstrations (*ibid* pp 444–454). Papers offered at the meeting of the American Association of Plastic Surgery at the Univ of Texas Medical Branch, Galveston, April 21–23, will appear in the *Journal of Plastic & Reconstructive Surgery*. The discussions at the Josiah Macy, Jr., Foundation Conference on "Medical & Psychological Team-work in the Care of the Chronically Ill," held at the Univ of Texas Medical Branch, Galveston, March 28–31, will appear in *Texas Reports on Biology & Medicine*, & separately in book-form.

GENERAL: E Bossi does well with mother-child relationship in *Child with a Flower* (Macmillan, NY, 54, 205pp, $3.75) . . . L Carmichael edits 2nd ed of *Manual of Child Psychology* (Wiley, NY, 54, 1295pp, $12) . . . G Carson revives *The Old Country Store* (Oxford Univ Press, NY, 54, 330pp, $5) . . . HS Commager clarifies *Freedom, Loyalty, Dissent* (Oxford Univ Press, NY 54, 143pp, $2.5) . . . PR Heyl says gravitation is still a mystery (*Sci Month* 78/303/54) . . . H Loewe describes long scientific collaboration of P Ehrlich (1854–1915) & E von Behring (1854–1917) (*Arzn-Forsch* 4/107/54) . . . AH Maslow discusses instinctoid nature of basic needs (*J Personal* 22/326/54) . . . DW Maurer & VH Vogel analyse *Narcotics & Narcotic Addiction* (CC Thomas, Springfield, Ill, 54, 320pp, $7.5) . . . VJ McGill goes into *Emotions & Reason* (CC Thomas, Springfield, Ill, 54, 122pp, $3.5) . . . E Olson analyses *The Poetry of Dylan Thomas* (Univ Chicago Press, 54,

164pp, $3.25) . . . RJ Pumphrey is witty on the evolution of thinking (*Brit J Philos Sci* 4/315/54) . . . E Quastler edits *Information Theory in Biology* (Univ Illinois Press, Urbana, 54, 234pp, $4) . . . G Sarton appraises *Galen of Pergamon* (Univ Kansas Press, Lawrence, 54, 124pp, $2.5) . . . FC Sheridan's *Galveston Island* (in 1839) is edited by WW Pratt (Univ Texas Press, Austin, 54, 223pp, $3.5) . . . LW Terman completes study of gifted children in *The Gifted Child Grows Up* (Stanford Univ Press, Stanford, Calif, 54, 448pp, $6) . . . P Tillich doubles in *Love, Power & Justice* (Oxford Univ Press, NY, 54, 118pp, $2.5) . . . & *The Courage to Be* (Yale Univ Press, New Haven, Conn, 54, 208pp, $3).

CLINICAL: FP Chinard & Co contribute symposium on nephrotic syndrome (*J Clin Invest* 33/621–723/54) . . . CN Frazer & IH Blank offer *A Formulary for External Therapy of the Skin* (CC Thomas, Springfield, Ill, 54, 118pp, $3) . . . FM Hanger discusses meaning of results of liver function tests (*Am J Med* 16/565/54) . . . HS Kaplan & SJ Robinson consider *Congenital Heart Disease* (McGraw-Hill, NY, 54, 126pp, $12.5) . . . M Kitaoka & Co indicate glutamyl-choline & thiamine may aid in polio (*Jap J Med Sci Biol* 6/475/53) . . . RJ Popkin notes subjective disability in post-phlebitic syndrome (*Calif Med* 80/291/54) . . . M Prinzmetal & Co continue studies on mechanism of ventricular action (*Am J Med* 16/469, 490/54) . . . W Raab & W Gigee find excretion of conjugated catechols decreased in uremia (*Circulat* 9/592/54) . . . S Sriranachari & MD Ananthachari indicate relation between malnutrition & liver cirrhosis (*Indian J Med Sci* 8/31/54).

PRECLINICAL: PK Bondy suggests pituitary growth hormone promotes fat use as metabolic substrate, aiding storage of protein & carbohydrate as protoplasm & glycogen (*Yale J Biol Med* 26/263/54) . . . AP Fishman finds constant respiratory dead space (av 164 ml) over wide range of conditions (*J Clin Invest* 33/469/54) . . . DL Fox writes on *Animal Biochromes & Structural Colours* (Cambridge Univ Press, NY, 54, 379pp, $11) . . . H Gest analyses oxidation of molecular hydrogen by microorganisms (*Bact Rev* 18/43/54) . . . G Hodgson & J Toha find hemorrhagic anemic plasma or urine increases red cell & hemoglobin production (*Blood* 9/299/54) . . . GB Koelle edits symposium on neurohumoral transmission (*Pharmacol Rev* 6/3–131/54) . . . BW Meade shows salicylic acid only of various hydroxybenzoic acids, increases oxygen consumption (*Ann Rheum Dis* 13/61/54) . . . B Nisman surveys LH Stickland reaction (*Biochem J* 28/1746/34) of coupled deamination (*Bact Rev* 18/16/54) . . . EL Oginsky & WW Umbreit offer *An Introduction to Bacterial Physiology* (WH Freeman, San Francisco, 54, 512pp, $6) . . . KH Schmidt considers mutagenic effects of triethylenephosphoramides & mercaptopurins (*Arzn-Forsch* 4/146/54).